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great pleasure to come in personal contact with Mr. Chazal, who made frequent visits to Dr. Shepard. It was Mr. Chazal's custom to arrive on the late afternoon train from Charleston, spend the night, and leave Summerville early the following morning. It was also the writer's good fortune to be present frequently during these visits or "talk festa", which lasted late into the night. These two men held each other in high esteem. Their conversations were usually confined to reminiscences of the phosphate industry in South Carolina, and many were the interesting stories they told in connection with its pioneer days. Although Dr. Shepard had severed his connections with the phosphate industry when he came to Summerville to attempt the establishment of a tea industry in America, he was still interested in hearing about the activities of his former industry, especially about the work in his own state and in the Shepard laboratory. In these discussions, Mr. Chazal's rugged honesty impressed the writer forcibly. He was a man of deep-rooted convictions and firmly believed that the longer and more arduous methods, such as those used in the Shepard laboratory, gave more reliable results. Therefore, when other chemical laboratories in the country adopted simpler and quicker methods of analysis, Mr. Chazal would not make the change, even at the expense of losing some of his patronage. He was a hard and conscientious worker and spent the greater part of his life in solving the many problems that arose in his chosen profession. Although Mr. Chazal was not a pioneer in the development of the phosphate industry in South Carolina, as were Dr. St. Julian Ravenel, Francis S. Holmes, and Dr. Shepard, he knew the details of all its branches as few men did.

G. F. MITCHELL.

It is with sorrow that we announce the death of Dr. J. K. Haywood, which occurred on November 30, 1928, at Emergency Hospital, Washington, D. C. Dr. Haywood was one of the active members of the Association of Official Agricultural Chemists and served as president during the year 1917.

An obituary will appear in a later issue of *This Journal*.

BOARD OF EDITORS.

PROCEEDINGS OF THE FORTY-FOURTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1928.

The forty-fourth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 29-31, 1928.

The meeting was called to order by the president, Oswald Schreiner, Washington, D. C., on the morning of October 29th, at 10 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1929.

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G. G. FRARY.

Recommendations of Referees.

(Figures in parentheses refer to year in which appointment expires.)

E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.), *Chairman*.

SUBCOMMITTEE A: A. G. McCall (1930), (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman*; R. N. Brackett (1932); H. H. Hanson (1934). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides (fluorine compounds); caustic poisons; soils and liming materials (reaction value of soils, liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, determination of moisture); sugars and sugar products (honey, maple products, starch conversion products; drying, densimetric, and refractometric methods; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, nitrogen activity methods in fertilizers, potash); plants (preparation of plant material for analysis, less common metals in plants, total chlorine in plants); paints, paint materials, and varnishes.]

SUBCOMMITTEE B: L. E. Warren (1930), (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*; H. C. Lythgoe (1932); A. G. Murray (1934). [Specific gravity and alcohol, spices and other condiments, naval stores (turpentine); drugs (crude drugs, radioactivity in drugs and water, laxatives and bitter tonics, mercurials, microchemical methods for alkaloids, terpin hydrate, santonin, ether, bioassay of drugs, fluidextract of ginger, ephedra, thymol, menthol, bromides-chlorides, oil of chenopodium, both salicylates and other phenols in mixtures, quantities of iodides in mixtures, bismuth compounds in tablet; beers, wines, and distilled liquors; colorimetric methods for vitamins.]

SUBCOMMITTEE C: H. A. Lepper (1930), (Food, Drug and Insecticide Administration, Washington, D. C.), *Chairman*; J. O. Clarke (1932); C. D. Howard (1934). Dairy products (milk, butter, cheese, malted milk, dried milk, ice cream, milk proteins, qualitative tests); fats and oils; baking powders and baking chemicals; eggs and egg products (total solids, fat, lipoids, and lipid P_2O_5 ; detection of decomposition; water-soluble protein, unsaponifiable matter, and ash); food preservatives, coloring matters in foods, metals in foods, fruits and fruit products (ash in fruit products, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins), gelatin, cacao products (crude fiber, cacao butter), cereal foods (sampling of flour, ash in flour and gasoline color value, glutenin in flour, hydrogen-ion concentration of flour, diastatic value of flour, starch in flour, flour-bleaching chemicals, methods for bread analysis—(a) sampling and determination of moisture, (b) lipoids and fat in bread, (c) milk solids in milk bread, (d) rye flour in rye bread—experimental baking tests, moisture in alimentary pastes, unsaponifiable matter in flour and in alimentary pastes and water-soluble protein in alimentary pastes).

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General referee: J. J. T. Graham, Food, Drug and Insecticide Adm., Washington, D. C.

FLUORINE COMPOUNDS:

Associate referee: G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.

CAUSTIC POISONS:

General referee: C. M. Smith, Food, Drug and Insecticide Adm., Washington, D. C.

SOILS AND LIMING MATERIALS:

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REACTION VALUE OF SOILS:

a. ALKALINE SOILS:

Associate referee:

b. ACID SOILS:

Associate referee: E. T. Wherry, Bureau of Chemistry and Soils, Washington, D. C.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

FEEDING STUFFS:

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Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:

Associate referee: H. A. Halvorson, Old Capitol Building, St. Paul, Minn.

DETERMINATION OF MOISTURE:

Associate referee: G. E. Grattan, Department of Agriculture, Ottawa, Can.

SUGARS AND SUGAR PRODUCTS:

General referee: R. T. Balch, Bureau of Chemistry and Soils, Washington, D. C.

HONEY:

Associate referee: H. A. Schuette, University of Wisconsin, Madison, Wis.

MAPLE PRODUCTS:

Associate referee: J. F. Snell, MacDonald College, Quebec, Canada.

STARCH CONVERSION PRODUCTS:

Associate referee:

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:

Associate referee: J. F. Brewster, Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS:

Associate referee: F. W. Zerban, N. Y. Sugar Trade Laboratory, New York City.

CHEMICAL METHODS FOR REDUCING SUGARS:

Associate referee: R. F. Jackson, Bureau of Standards, Washington, D. C.

FERTILIZERS:

General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:

Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:

Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

NITROGEN ACTIVITY METHODS IN FERTILIZERS:

Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH:

Associate referee: L. D. Haigh, Agricultural Experiment Station, Columbia, Mo.

PLANTS:

General referee: O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS:

Associate referee: H. R. Kraybill, Agricultural Experiment Station, Purdue, Ind.

LESS COMMON METALS IN PLANTS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE IN PLANTS:

Associate referee: M. F. Mason, Agricultural Experiment Station, E. Lansing, Mich.

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General referee: C. S. Ladd, Office of Food Commissioner and Chemist, Bismarck, N. D.

SPECIFIC GRAVITY AND ALCOHOL:

General referee: A. W. Hanson, Food, Drug and Insecticide Adm., Minneapolis, Minn.

SPICES AND OTHER CONDIMENTS:

General referee: K. C. Beeson, State Chemical Laboratory, Vermilion, S. D.

NAVAL STORES:

General referee: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D.C.

TURPENTINE:

Associate referee: V. E. Grotlisch, Food, Drug and Insecticide Adm., Washington, D. C.

DRUGS:

General referee: A. E. Paul, 1625 Transportation Bldg., Chicago, Ill.

CRUDE DRUGS:

Associate referee: H. W. Youngken, Massachusetts College of Pharmacy, Boston, Mass.

RADIOACTIVITY IN DRUGS AND WATER:

Associate referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

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MERCURIALS:

Associate referee: R. S. Roe, Food, Drug and Insecticide Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR ALKALOIDS:

Associate referee: C. K. Glycart, Food, Drug and Insecticide Adm., Chicago, Ill.

TERPIN HYDRATE:

Associate referee: H. R. Watkins, Food, Drug and Insecticide Adm., Washington, D. C.

SANTONIN:

Associate referee: H. M. Burlage, Oregon Board of Pharmacy, Corvallis, Ore.

ETHER:

Associate referee: W. F. Kunke, Food, Drug and Insecticide Adm., Chicago, Ill.

BIOASSAY OF DRUGS:

Associate referee: W. T. McClosky, Food, Drug and Insecticide Adm., Washington, D. C.

FLUID EXTRACT OF GINGER:

Associate referee: H. C. Lythgoe, Department of Public Health, Boston, Mass.

EPHEDRA:

Associate referees: C. K. Glycart and A. E. Paul.

THYMOL:

Associate referee: F. L. Hart, Food, Drug and Insecticide Adm., Chicago, Ill.

MENTHOL:

Associate referee: F. L. Elliott, Food, Drug and Insecticide Adm., Baltimore, Md.

BROMIDES-CHLORIDES:

Associate referee: H. Wales, Food, Drug and Insecticide Adm., Washington, D. C.

OIL OF CHENOPODIUM:

Associate referee: L. B. Broughton, College Park, Md.

DETERMINATION OF BOTH SALICYLATES AND OTHER PHENOLS IN MIXTURES:

Associate referee: F. C. Synkovich, Food, Drug and Insecticide Adm., Chicago, Ill.

DETERMINATION OF SMALL QUANTITIES OF IODIDES IN MIXTURES:

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BISMUTH COMPOUNDS IN TABLETS:

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COLORIMETRIC METHODS FOR VITAMINS:

Associate referee: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

DAIRY PRODUCTS:

General referee: H. C. Lythgoe, Department of Public Health, Boston, Mass.

MILK:

Associate referee: H. Hoffmann, Jr., Dairy and Food Department, Minneapolis, Minn.

BUTTER:

Associate referee: H. Hoffmann, Jr.

CHEESE:

Associate referee: E. O. Huebner, Dairy and Food Commission, Madison, Wis.

MALTED MILK:

Associate referee: B. G. Hartmann, Food, Drug and Insecticide Adm., Washington, D. C.

DRIED MILK:

Associate referee: E. L. P. Treuthardt, Food, Drug and Insecticide Adm., Boston, Mass.

ICE CREAM:

Associate referee: R. O. Baird, Food and Drug Laboratory, Bismark, N. Dak.

MILK PROTEINS:

Associate referee: H. C. Waterman, Office of Experiment Stations, Washington, D. C.

QUALITATIVE TESTS:

Associate referee: Philip H. Smith, Feed and Seed Control, Amherst, Mass.

FATS AND OILS:

General referee: G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

BAKING POWDERS AND BAKING CHEMICALS:

General referee: G. L. Bidwell, Food, Drug and Insecticide Adm., Washington, D. C.

EGGS AND EGG PRODUCTS:

General referee: Samuel Alfend, Food, Drug and Insecticide Adm., St. Louis, Mo.

TOTAL SOLIDS, FAT, LIPOIDS, LIPOID P_2O_5 :

Associate referee:

DETECTION OF DECOMPOSITION:

Associate referee: H. I. Macomber, Food, Drug and Insecticide Adm., New York City.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH:

Associate referee: Samuel Alfend.

FOOD PRESERVATIVES:

General referee: W. W. Randall, State Department of Health, Baltimore, Md.

COLORING MATTERS IN FOODS:

General referee: C. F. Jablonski, Food, Drug and Insecticide Adm., New York City.

METALS IN FOODS:

General referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

FRUITS AND FRUIT PRODUCTS:

General referee: H. J. Wichmann, Food, Drug and Insecticide Adm., San Francisco, Calif.

ASH IN FRUIT PRODUCTS:

Associate referee: V. B. Bonney, Food, Drug and Insecticide Adm., Washington, D. C.

FRUIT ACIDS:

Associate referee: B. G. Hartmann, Food, Drug and Insecticide Adm., Washington, D. C.

CANNED FOODS:

General referee: E. K. Nelson, Bureau of Chemistry and Soils, Washington, D. C.

VINEGARS:

General referee: A. M. Henry, Food, Drug and Insecticide Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

General referee: J. W. Sale, Food, Drug and Insecticide Adm., Washington, D. C.

MEAT AND MEAT PRODUCTS:

General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:

Associate referee: W. S. Ritchie, University of Missouri, Columbia, Mo.

GELATIN:

General referee: R. M. Mehurin, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:

General referee: J. O. Clarke, Food, Drug and Insecticide Adm., New York, N. Y.

CRUDE FIBER:

Associate referee: Marie L. Offutt, Food, Drug and Insecticide Adm., New York, N. Y.

CACAO BUTTER:

Associate referee: M. M. Jackson, Food, Drug and Insecticide Adm., New York, N. Y.

CEREAL FOODS:

General referee: J. A. Le Clerc, Bureau of Chemistry and Soils, Washington, D. C.

SAMPLING OF FLOUR:

Associate referee: H. Runkel, Food, Drug and Insecticide Adm., Chicago, Ill.

ASH IN FLOUR AND GASOLINE COLOR VALUE:

Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

GLUTENIN IN FLOUR:

Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

HYDROGEN-ION CONCENTRATION OF FLOUR:

Associate referee: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

DIASTATIC VALUE OF FLOUR:

Associate referee:

STARCH IN FLOUR:

Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

FLOUR-BLEACHING CHEMICALS:

Associate referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

METHODS FOR BREAD ANALYSIS:**a. SAMPLING AND DETERMINATION OF MOISTURE:**

Associate referee: L. H. Bailey.

b. LIPOIDS AND FAT IN BREAD:

Associate referee: Samuel Alfend, Food, Drug and Insecticide Adm., St. Louis, Mo.

c. MILK SOLIDS IN MILK BREAD:

Associate referee: L. H. Bailey.

d. RYE FLOUR IN RYE BREAD:

Associate referee: J. B. Reed, Food, Drug and Insecticide Adm., Washington, D. C.

EXPERIMENTAL BAKING TESTS:

Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

MOISTURE IN ALIMENTARY PASTES:

Associate referee: S. C. Rowe, Food, Drug and Insecticide Adm., Washington, D. C.

UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN IN ALIMENTARY PASTES:

Associate referee: Samuel Alfend, Food, Drug and Insecticide Adm., St. Louis, Mo.

BEERS, WINES AND DISTILLED LIQUORS:

General referee: W. V. Linder, Bureau of Internal Revenue, Washington, D. C.

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PRESIDENT'S ADDRESS.¹

THE RÔLE OF THE RARER ELEMENTS IN SOILS, FOODS, AND DRUGS.

**By OSWALD SCHREINER (Division of Soil Fertility, Bureau of Chemistry
and Soils, Washington, D. C.).**

For over half a century, since Knop and others showed conclusively that potassium, phosphorus, nitrogen, calcium, magnesium, sulfur, carbon, hydrogen, oxygen, and iron were essential to plant growth in water culture, we have been taught and have been teaching that only these ten elements were necessary for growth and maturation of our agricultural crops. In fertilizer practice, to increase crop growth, we have contented ourselves chiefly with the application of phosphorus, potassium, and nitrogen to our soils, with lime to correct soil acidity and not as plant food, on the assumption that soils, fertilizers, and manures supply sufficient of the other mineral elements for profitable crop production. Yet the researches of Garner, Gile, Reimer, and others have shown us strikingly that magnesium, iron, and sulfur deficiencies can exist in comparatively large soil areas. Similarly, we have been able to show a marked phosphorus deficiency in some western soil regions devoted to sugar-beet culture, so that the application of even small amounts of this element produces large increases in sugar-beet production, as shown by Hurst.

In testing, perfecting, and standardizing our official methods we likewise have confined ourselves in the years that are past chiefly to this limited number of important elements and their compounds. Research on plant and animal nutrition, however, has reached such a stage of progress that we must now move on to the testing, perfecting, and standardizing of an entirely new type of methods. In these, small quantities, and other and rarer elements will be involved, together with the speed and precision demanded by the progress of the time in scientific research, in industrial processes, and in control measures.

It is quite natural that the agricultural chemists and the plant and animal physiologists should devote first attention to those elements present in plants and animals in largest amount, but with the development of research in analytical chemistry and with more delicate and refined methods and apparatus, it is to be expected that the less common elements, considered for convenience sake as nonessential, will be found to be most important factors in plant and animal nutrition, metabolism, and health. I am referring especially to the functions which elements like manganese, copper, boron, iodine, zinc, arsenic, barium, strontium,

¹ Presented Tuesday morning, October 30th, as special order of business for 11 o'clock.

caesium, titanium, chromium, vanadium, aluminum, and silicon play in the newer research in plant and animal physiology.

The nutrition studies of Reed and Haas on citrus trees grown in water cultures illustrate the absolute necessity of the presence of some of these rarer elements, although they did not determine which of them were specific in remedying the observed physiological disturbances. With all usual plant foods present in complete culture solutions they observed normal growth at first, but sooner or later serious symptoms of malnutrition developed. The orange leaves recurved or rolled strongly, and

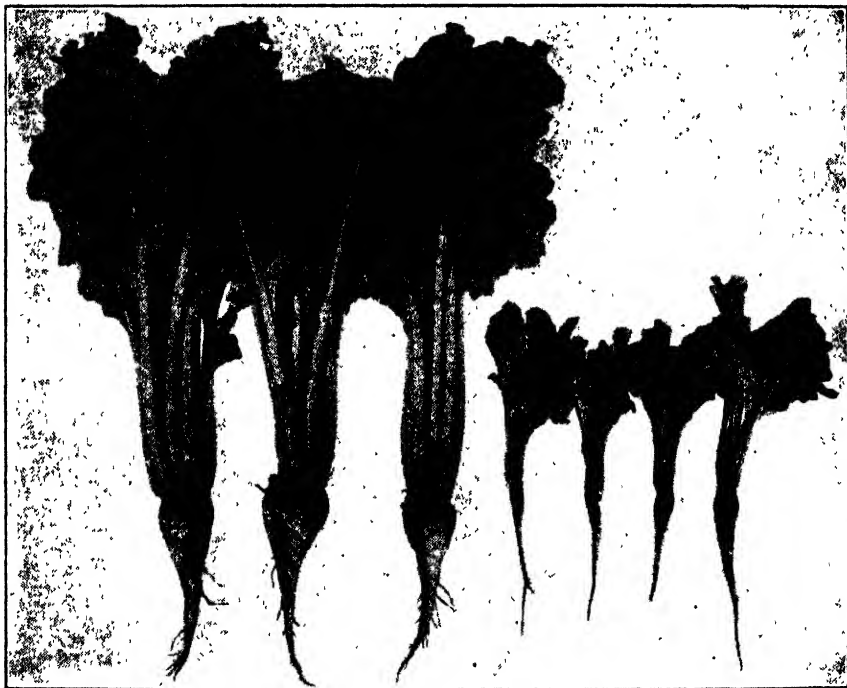


FIG 1. THE EFFECT OF SMALL QUANTITIES OF PHOSPHATE ON SUGAR BEETS IN A COLORADO SOIL.

the midribs thickened and frequently split open. The leaves showed yellowish spots whose centers often had resinous exudations, and the shoots frequently showed the formation of multiple buds, symptoms which are usually associated with the disease known as dieback in citrus orchards. All these physiological disturbances disappeared on treatment with a mixture of the salts of the less common elements, aluminum, iodine, titanium, bromine, strontium, lithium, manganese and boron.

Experimenters using water cultures in nutrition studies have not usually worked with trees growing for several years but have used shorter-lived crops such as cereals, and hence have not noted such well developed symptoms as in this case with orange trees for the following

reasons: (1) the short time of experimentation; (2) sufficient of the rarer elements in the seeds; (3) inevitable impurities in the chemicals used; and (4) solubility of the containers, which may have supplied sufficient quantities of these rarer elements for short periods of growth since only minute quantities are required.

During this same period of half a century or more in which we have been chiefly concerned with the determination and analysis of the ten important elements of plant and animal tissues, research work has been quietly going on to determine the effect on plants of these less common elements, especially manganese, until today some of these elements are actually used in practical agriculture with signal success. Passing over the early work of Bertrand, of Brenchley, and of our own—on the presence of manganese in the enzyme laccase, the growth effect of manganese in water cultures, the oxidation in soil extracts and crop growth in field soils—as well as numerous other researches involving manganese as a beneficial or a toxic element in plant growth, I wish to refer briefly, in illustration, to the fundamental work of McHargue in Kentucky and our own practical tests on soils at the Arlington Farm, Virginia, and in southern Florida by Skinner and by Dawson.

The painstaking work of McHargue with manganese in controlled solution cultures shows conclusively that it must be considered one of the elements essential to plant growth. He also reaches the conclusion that not only manganese, but other elements which he has shown to be present in the germs and pericarps of seeds, in the leaves of plants and in Kentucky bluegrass especially, such as copper, zinc, nickel and cobalt, play a part in the vitamin cycle in nature, and that they aid plants in synthesizing organic combinations which function as catalysts, enzymes and vitamins and in turn, upon entering the animal system as food, are resynthesized into hormones, oxidizing and catalyzing enzymes, the animal vitamins so essential to proper growth and function.

Considering our own work, following the early reports by Bertrand, we published first in 1910, the influence of manganese on soil oxidation and the beneficial effect of this element in poor soil extracts, whose oxidative power was at the same time increased. The results of field tests inaugurated earlier at the Arlington Experiment Farm of the Department in 1907 and continued for six years were published in 1914. This soil, of an acid nature, gave very inconsistent results over the six year period. The only conclusion that could be drawn was that manganese applied at the rate of 50 pounds of manganese sulfate per acre was not beneficial to the wheat, rye, cowpeas, corn and potatoes grown on this acid soil.

Subsequently, these same manganese plots were kept neutral or slightly alkaline by the addition of lime with the result, published in 1916, that the oxidative power of the neutralized soil was increased by manganese, and the productivity was increased in the case of wheat, rye, timothy, beans, corn and cowpeas, while with potatoes no increase was observed.



FIG. 2. THE EFFECT OF SMALL QUANTITIES OF MANGANESE ON THE GROWTH AND FRUITING OF TOMATOES ON A CALCAREOUS SOIL FROM SOUTHERN FLORIDA.

We drew the conclusion that no profitable⁷ return is to be expected from manganese applications to soils of a persistent acid tendency until such soils are limed. This non-action on the part of manganese under acid conditions probably explains the many inconsistent reports of its action as found in the literature, and is confirmed in field practice by some recent results we have obtained under similar acid conditions in Eastern North Carolina, where manganese proved harmful unless lime was also

applied, and by some results under alkaline conditions in a highly calcareous soil region in Southern Florida where manganese has shown marked effects, and where it is now used with fertilizers in practical agriculture. The latter is a splendid illustration of the practical significance of these rarer elements, and I shall, therefore, describe this work briefly.

South of Miami, Florida, there are large areas that have been in the past, and are yet, periodically covered with water each year. This soil, composed almost entirely of calcium carbonate deposited from the sea water, contains also from 5 to 10 per cent organic matter, small amounts of the usual plant foods, a little silica, traces of iron and a few other minerals with manganese barely discernible by delicate tests. These areas, known as Glades, are being used largely for the production of tomatoes. The manner of growing tomatoes is as follows: as soon as these level fields permit cultivation after the water has receded in the late fall, the growers mark off rows and drop tomato plants a certain distance apart and throw on a handful of manure. This has been the only method that has proved successful. When the manure application is large and continuous, year in and year out, tomatoes can be grown successfully. If, on the other hand, no manure is used or the soil is stirred up, as is ordinarily done in other regions by plowing or cultivating, the tomatoes will not grow well and no amount of fertilizer helps the situation.

Analysis of the soil disclosed the fact that the difficulty was a lack or a deficiency of manganese. An examination of the manure showed that it was supplying the manganese. The manure comes from the Mississippi River Valley and other points in the United States and carries the manganese which the forage plants have absorbed by growing on soils in regions where this element is common.

We, therefore, attempted to determine whether manganese would replace the manure, and this has been on the whole successfully proved. Tomatoes can now be grown in Florida with manganese and fertilizer salts alone, without manure, or peat can be substituted for the manure by treating the peat with manganese, but the manganese is the keynote to the successful cultivation of tomatoes in these calcareous soils of southern Florida.

A chlorotic condition of the foliage manifested itself in white spots and areas between the veins. This could not be remedied with liberal applications of ordinary fertilizer salts, but the addition of minute quantities of manganese—50 pounds of manganese sulfate per acre, with an acre foot of soil weighing about four million pounds—produced strong, vigorous plants, deep green in color, luxuriant blossoming, and greatly increased fruit production. In fact, without manganese there is no fruit production, and the plants soon fade and die.

These experiments demonstrate beyond doubt that, whatever the

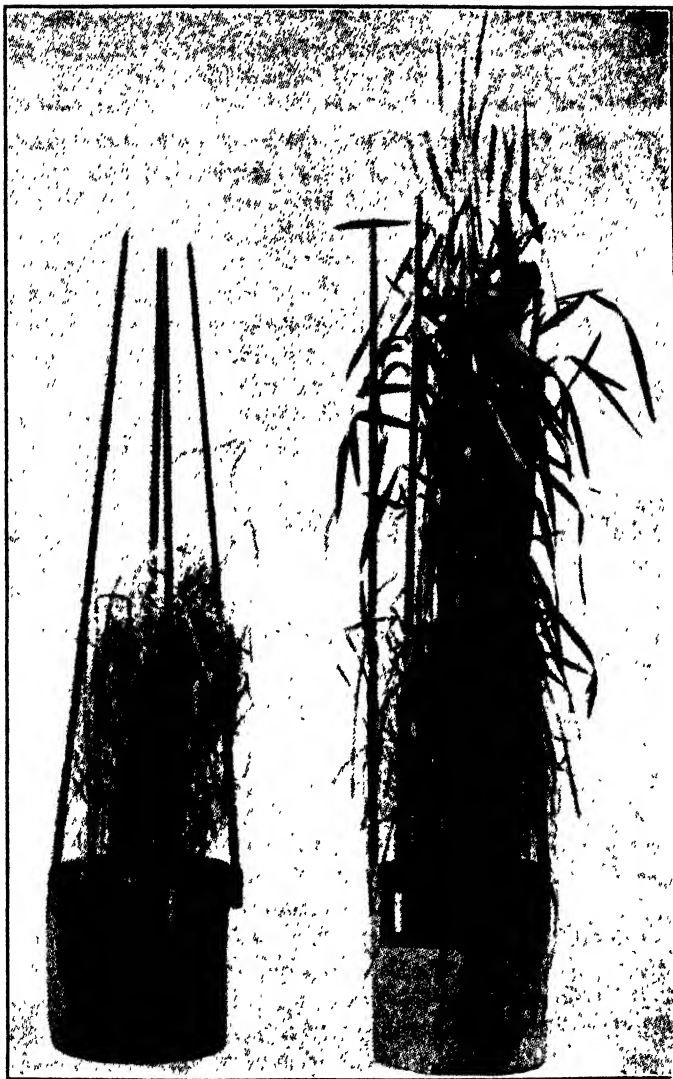


FIG. 3. THE EFFECT OF MANGANESE ON WHEAT (AFTER MCHARGUE).

fundamental function of manganese may be, the element is indispensable to the normal growth of the tomato plant under the conditions prevailing in this calcareous soil. Although the soil is of a unique type, owing to its exceedingly high calcium carbonate content, and is exceptional in possessing a negligible manganese content as compared with the general run of American soils, it nevertheless furnishes a striking illustration of the kind of problem that may arise more frequently in the future. With the utilization of modern pure fertilizer materials of chemical manufac-

ture, the problem may be extended to include not only manganese, but also such elements as zinc, copper, nickel, boron, etc., the relation of which to plant growth is not yet so fully understood.

The older fertilizers, consisting of plant and animal by-products, and even the inorganic fertilizer salts originating in natural deposits and containing greater or less amounts of accompanying impurities, are increasingly being supplanted by manufactured products of a high degree of purity, especially the air-derived nitrogen products. The application of these chemical fertilizer substances to the general run of soils containing sufficient reserves of the lesser inorganic constituents is not likely to involve any problem of deficiency. On the other hand, in soils where the amount of these less common constituents may be small or unavailable, deficiency will probably be noticed in time if pure chemicals only are used. In the problem described above the heavy applications of inorganic fertilizers, while furnishing an adequate supply of the major nutrients, could not meet the deficiency of manganese in the soil, but the manure procured from soil regions well supplied with manganese apparently carried sufficient quantities to supply the demands of the plant.

Hence, further fertilizer practice must take into consideration the rôle of manganese and other elements which may be proved indispensable to plant growth and function. Provision must also be made for determining deficiencies and meeting them through appropriate supplements to the fertilizers applied. At the same time it should be emphasized that the amounts of the constituents required are exceedingly small and that precautions must be exercised to prevent the toxic effects produced by excessive applications to soils and additions to fertilizers already containing amounts sufficient for plant requirements.

Florida furnishes another striking illustration of the effect of minute quantities of the rarer elements, especially of copper, in the work of Allison on Everglades peat. These peat lands present many problems and among them has been the lack of response to fertilizers. Allison has obtained plant growth responses on a long list of plants by using such unusual fertilizing elements as zinc, antimony, nickel, tin, barium, copper, and manganese. Of these the most favorable results were obtained with copper and the residual effects of this treatment have carried over for more than a single cropping season. By the use of copper, plant growth has been enormously stimulated, and although the problem is not yet solved, this research by Allison holds out much promise for the future of the Everglades. He also obtained good results with manure, but suggests that this may have been due to small quantities of these special elements contained in the manure in available form.

Interesting in this connection is also the now common practice in Florida of applying copper sulfate to citrus trees suffering from dieback, which is considered a physiological disease. As we have stated, the

symptoms of dieback are many, among them chlorosis or mottled leaf, gum pockets in the new shoots, and multiple buds, similar to those observed by Reed and Haas with incomplete culture media. Originally the crystals of copper sulfate were inserted under the bark with only moderate success, but for the last ten years or more the copper sulfate has been applied in fertilizers or separately to the soil. Beneficial results are obtained often enough to encourage citrus growers in the practice of using copper sulfate as a cure for dieback.



FIG. 4. THE EFFECT OF A SMALL QUANTITY OF COPPER ON VELVET BEAN GROWN IN EVERGLADES PEAT (AFTER ALLISON).

While it is obviously impossible to review all the conflicting evidence in the literature on this question of essentialness of the rarer elements in plants and foods, attention should nevertheless be called in passing to the striking results which Brenchley and Warington have obtained on the action of minute quantities of boron on the development of legumes, and to the work of Lipman and Sommers with zinc, boron, silicon and aluminum, which seems to show that minute quantities of these elements are necessary for proper development in a number of plants.

In connection with the discussion of the necessity of these rarer elements in culture media for plants and their importance in human foods,

it becomes also of interest to note that the value of sea food has been recognized since time immemorial. So well was it recognized that the use of sea food became an early adjunct of religious custom. It has been stated that sea water contains at least thirty-four of the chemical elements. It becomes, therefore, one of the best natural complete culture media we possess and in it develops three-fourths of the living vegetable and animal matter of the globe. This culture medium, which sustains most of the life of the world, contains a little of everything needed for life and through its mobile character remains constant in its composition. The soil, too, from which we humans secure most of our sustenance is in large measure made up of the detritus of the globe, but unlike the mobile sea the land is relatively fixed, and moreover the soil is only too often the less soluble residue, left after the waters of the globe have broken down the rock and transported the particles from place to place. Nevertheless, the chemist's skill, though still hampered by the lack of methods for determining small quantities, has been able to disclose in soils and plants the presence of a very large number of common and rare elements, but their universality of occurrence is in the nature of the case not so great as in the mobile sea.

Likewise, the animal body has been compared to a miniature cosmos in which all elements of the universe are found. However that may be, certain it is that the body of the human species contains, be it in large or infinitesimal quantities, practically all the known elements. The human species has perhaps the greatest power to move from place to place on our globe, over land and sea and through the air, secures its food from the most varied soil conditions of the world through a well developed civilization based on commercial intercourse, and partakes in addition largely of the foods of the ocean in which, as stated, the elements, both common and rare, are widely and uniformly distributed.

Plants, and especially our crop plants, are not so fortunately situated. These, in a measure, are compelled to obtain their sustenance from the soil on which they happen to have their birth, where they will live and multiply, and to which they will again return, upon their death, the elements borrowed for their existence. Hence, in certain regions, these rarer though vitally necessary elements of life may continue to be present in minimum quantities and, according to the law of the minimum, limit development and growth both of the plant and of animals and humans whose unfortunate lot it may be to subsist largely upon local products. Lack of lime and phosphate is a case in point. Only where calcium and phosphate are plentiful can forage crops be grown of such composition that they will produce strong, healthy, well-boned animals.

Lack of iodine in soils and crops is another illustration. The exhaustive work of Fellenberg on the iodine survey of the soils, waters and foods of Switzerland in its relation to the dreaded goiter in man is most commendable, and no less important are the researches conducted by



FIG. 5. THE EFFECT OF TRACES OF BORON ON THE BROAD BEAN (AFTER BRENCHELEY AND WARINGTON).

McClendon and other American workers into the situation in our own glaciated regions where goiter is prevalent, and abortion in cattle and hairless young pigs the rule.

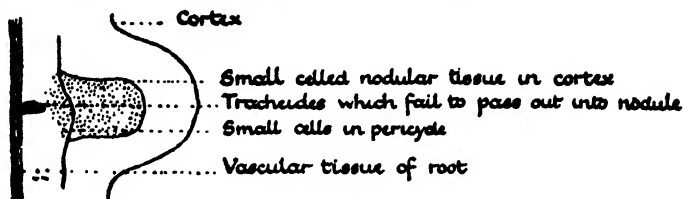
Iodine, of which the human body contains only about 25 milligrams (approximately $\frac{1}{4}$ of a grain) largely localized in the thyroid gland, is vital to health. If, for any reason, this necessary minimum is diminished, serious disorders result, among them goiter, as is the case in our glaciated districts, covering roughly the northern half of the United States and

especially the mountain regions. It has been shown that iodine occurs in many foods, especially in normal milk and sea foods, and that so little as 14 γ (fourteen thousandth part of a milligram) in the daily food is an ample supply for human beings. It can also be supplied as mineral iodides in drinking water and table salts, but the organic forms in foods are preferred. Kelp, or sea weed, having concentrated the iodine from the sea water in which it grew, has lately been the subject of experimentation as a cattle food. Thus Cavanaugh, experimenting on the dairy herd of Mrs. McCormick of Illinois, has recently shown that the iodine content of the milk could be materially increased in this manner. Of further importance to us, as agricultural chemists, is the fact that iodine occurs in some of the salt deposits used as fertilizers, notably so in sodium nitrate. Whether this iodine in fertilizer is of economic importance is a debatable point upon which we need more evidence. According to von Wrangell the iodine does not increase the yield of plants sufficiently to warrant its addition to fertilizers in goiter-affected regions, and it is better to rely upon the selection of suitable foodstuffs to supply the necessary iodine for body use.

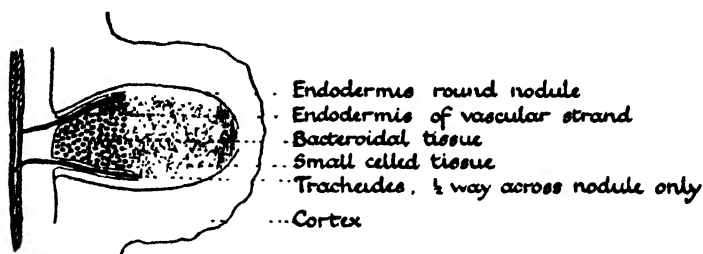
Another one of the rarer elements of great importance is fluorine, and for this we need more accurate and ready methods of determination. Fluorine appears to be of fundamental importance in the formation of the enamel of the teeth in growing children, and as you so well know, good teeth are fundamental to good health. Fluorine occurs in soils and plants and in fertilizers and there is some evidence that it plays a part in crop production, but we need more evidence based on accurate experimentation with precise methods of determination before this point is definitely settled. Similarly chromium, vanadium and titanium have been found in soils and plants and their effect on plant growth has been studied, but the story of their action is not yet clear, and the biological experimenter is still awaiting the chemist's helpful guidance with methods for ready and accurate determinations of such elements.

The further need for methods that will determine small quantities is well illustrated by the fact that these rarer elements are often found only in specific parts of the plant and animal and therefore only a limited amount of material can be available for study. These methods must also be fairly rapid as well as accurate and trustworthy, because it is necessary to make many explorative tests and investigations to determine properly what the function of these rarer elements may be. In his work in connection with root rot in corn, Hoffer has shown that iron and aluminum tend to accumulate in certain tissues at the nodes. McHargue and others have shown that such metals as copper, manganese and zinc occur principally in the germ of such grains as wheat and corn. Branchley has convincingly shown the influence of boron on legumes in developing the vascular system of the nodule and in aiding the exchange of nutrients between bacteria and plant, thus being vital to nitrogen fixation. What

No Boron. Type I. Abortive or undeveloped nodule



Type II Incompletely developed nodule



With Boron. Fully developed nodule

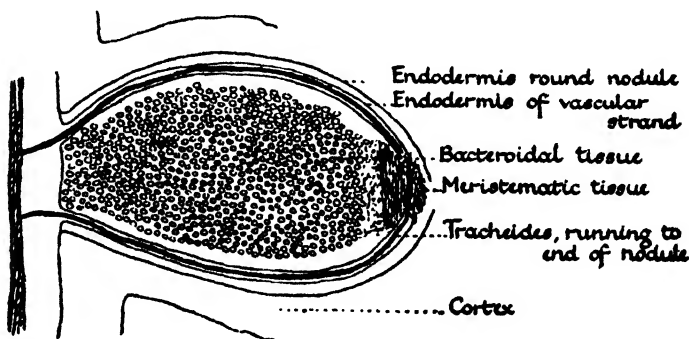


FIG. 6. SHOWING THE EFFECT OF BORON ON THE PRODUCTION OF THE VASCULAR SYSTEM OF THE LEGUME NODULE WHICH ENABLES THE PLANT TO OBTAIN NITROGENOUS MATTER FROM THE NODULE AND IN RETURN SUPPLY SUGARS AND OTHER FOOD SUBSTANCES TO THE NITROGEN-FIXING BACTERIA IN THE NODULE (AFTER BRENCHELEY).

Brenchley has not shown is whether boron is specifically located in the nodule and for the very good reason that such quantities as are required are not readily determinable by present chemical methods. Similarly, the amounts of boron which cause damage to citrus trees in California, as Kelley has so conclusively shown, and the amounts of boron which adhere to fruit treated for molds and rots, cannot be accurately deter-

mined with present methods. The use of arsenic and of copper, lead and other metals in sprays and the legal limits imposed on fruits sent abroad as well as the desirability of knowing the quantities adhering to fruits and vegetables for home consumption make methods for the ready estimation of these elements desirable and necessary.

During the first half of the nineteenth century our chemical concept was practically identical with that of chemical analysis. After Lavoisier had established the weight relationships which existed when chemical reactions took place, every one sought to determine the composition of unknown bodies by means of the balance. Every chemist was of necessity also an analyst, and the great chemists of that day—Berzelius, Stas, Wöhler, Bunsen, Gay-Lussac, Vauquelin, Dumas—are famous as analysts. The second half of the nineteenth century changed this picture and the wonderful development of organic chemistry found the disciples of this branch of science following synthetic rather than analytic methods, and only that branch of elementary analysis closely associated with the chemistry of the carbon atom received attention. Thus developed gradually the erroneous impression that analytical chemistry was only a means to an end and not in itself worthy of high research endeavor. This condition changed again in the latter half of the century, when it was recognized that the newer teachings and theories of general chemistry could also be applied to chemical analysis, with the result that new methods and new apparatus were applied to furthering the research in this field.

The brilliant work of Bunsen and Kirchoff in spectral analysis gave us an unexpected insight into the structure of the elements and made possible the determination of quantities too minute for the most accurate balance. Modern developments in the spectrophotometric field and in Röntgen ray spectras give us a still greater insight into the structure of matter and open up fields of research of the greatest promise. Electrical conductivity and electrometric analysis are further illustrations of the progress of analytical procedure in the light of modern research.

For the determination of very small quantities of matter, colorimetry has been of the greatest service, together with the turbidity measurements by nephelometric methods. The electroscope has been invaluable in the measurements of radioactivity, and the microscope with its refraction measurements of minute particles of matter is a veritable boon to the modern analyst. Analysis and research go hand in hand and who can say which is the master and which the handmaiden?

Microchemical analysis is the order of the day, for we must conserve energy, time and material in modern research. Not only the chemist, but the pathologist and the biologist demand new methods in solving the perplexing problems that confront them in their research work. Only recently I had a request from a noted biologist for a method to determine the nitrogen content of a certain organ of one of the micro-



FIG. 7. THE EFFECT OF SMALL QUANTITIES OF ZINC ON SUNFLOWER (AFTER SOMMER).

scopic nemas. With rare skill he was able to separate this nema from its host and then with still rarer skill to dissect from the nema the organ in question, the function of which he surmised might be solved by a nitrogen determination. With untiring patience he could furnish a number of such organs aggregating perhaps as much as a milligram of wet weight, possibly a tenth of a milligram of dry weight. Here was a challenge to the chemist from the biologist and even microchemical methods seemed inadequate and crude. The biologist had accomplished his task

with rare skill but the most refined chemical methods failed him in the furthering of his research. If we do not supply the biological and medical research worker with tried and tested methods, he will devise them himself and perhaps do incalculable damage to science through the unsuspected inaccuracies involved. The literature is already overloaded with observations on hydrogen-ion concentrations and conclusions based on inadequate methods which it will take years to eliminate.

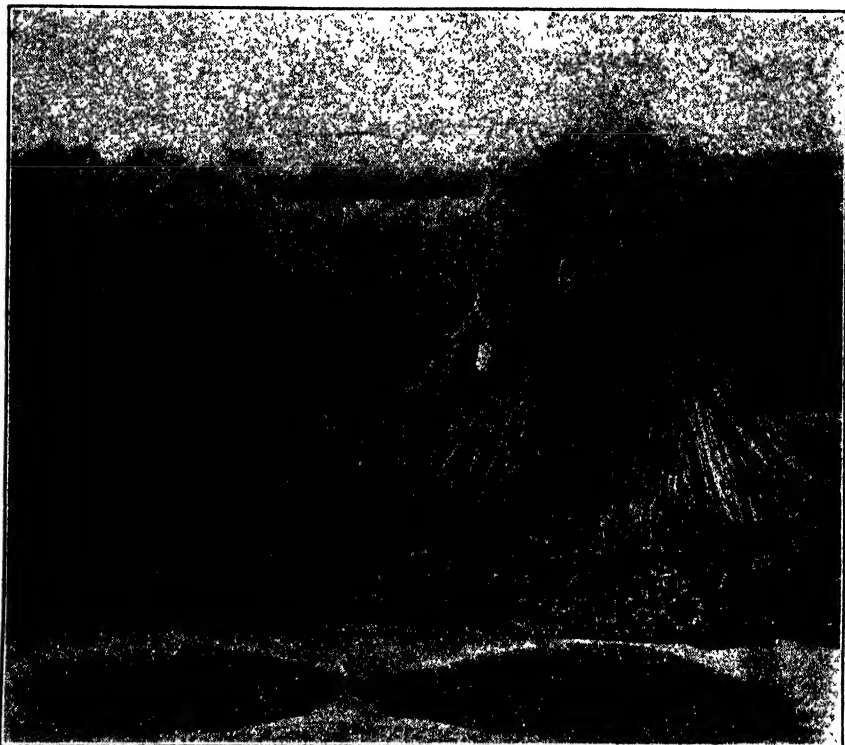


FIG. 8. THE EFFECT OF MANGANESE ON THE GROWTH AND YIELD OF WHEAT ON A WELL-LIMED SOIL.

This association, through its publication, *Methods of Analysis*, which is based on long years of painstaking work of its members, has rendered a signal service to the welfare of the people of this country and of the world. These methods deal more especially with commoner elements in soils, fertilizers, feeding stuffs, human foods and drugs, to the end that the public welfare and health be safe-guarded. We may well feel proud of the success achieved, but we must look forward into new fields of achievement and service. To me this task seems to comprise developing and testing the new and rapid methods needed in industrial and scientific research for the accurate determination of small quantities of the less common elements known to play no small part in industrial processes and in the life of animals and plants.

ORDER OF PUBLICATION.

The reports of the committee presented on the last day of the annual meeting are given at the beginning of the proceedings rather than in their chronological order. This will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY.

WEDNESDAY—MORNING SESSION.

REPORT OF THE REPRESENTATIVE AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The seventh annual meeting of the National Conference on Pharmaceutical Research was held at the Eastland Hotel, Portland, Me., August 18, 1928. The roll call showed delegates present from 15 affiliated organizations. The total attendance numbered about 60.

The following items of interest were selected from the reports of the chairmen of the 13 standing committees and the delegates of the 15 affiliated organizations.

In his report on galenicals E. F. Cook stated, among other things, that historical investigation showed that 23 of the present-day galenicals were of American origin.

H. W. Youngken, Chairman of the Committee on Sources and Identification of Botanic Drugs, reported that *Oncoba echinata* (*Flacourtiaceae*) had been found to yield an oil quite similar to the oil obtained from *Taraktogenos Kurzii* and that this oil is used by the natives of West Africa in the treatment of skin diseases. He also stated that ephedrine and pseudoephedrine occur in various species of ephedra growing in China and that an alkaloid with properties resembling emetine but much less toxic was obtained from a species of *holorrhena*. Special mention was made of the forthcoming report to be published in the *Journal of the American Pharmaceutical Association* on the present commercial sources of American medicinal plants with exhaustive studies of such plants in Florida and New Jersey. It was also noted that commercial sandalwood oil has been found to be adulterated with castor oil.

E. N. Gathercoal reported on the standardization of botanic drugs. Special mention was made of the New York Laboratory method for the valuation of benzoin and G. L. Keenan's work on the optical identification of alkaloids. Attention was called to Fisher's work on the ash of vegetable drugs. The acid-soluble and acid-insoluble ash for a number of such drugs was given. Color standards for solutions and for crude drugs were recommended.

P. S. Pittenger, Chairman of the Committee on Pharmacology and Bioassays, reported that collaborative work on digitalis, ergot and vitamins A, B, C, and D would be undertaken during the coming year.

Methods of facilitating the publication of bibliographies and extensive reports of special investigations, such as the bibliography on solanaceous drugs by F. B. Kilmer and A. J. Hill's investigation of local anesthetics, were discussed.

The National Conference on Pharmaceutical Research was organized at an informal meeting of research workers at Cleveland, Ohio, in 1922. The membership of the conference was vested in certain specified organizations. The growth of the conference, involving increased expenses, led to the appointment of a Committee on Expansion at the 1927 annual meeting. The report of this committee recommended changes in the constitution and by-laws to provide a budget of \$2,500 and the employment of a full-time secretary. After two hours of discussion by the conference sitting as a committee of the whole it was decided to refer the matter back to the Committee on Expansion for report at the annual meeting in 1929.

On behalf of the Association of Official Agricultural Chemists 21 pages of galley proof, constituting the reports on drugs presented by the referees and associate referees at the annual meeting of the association in 1927, were exhibited.

H. R. WATKINS.

Approved.

No general report of the Committee on Editing Methods of Analysis was given, as no appointment was made to fill the vacancy caused by the resignation of R. W. Balcom, chairman of the committee.

The following report was compiled by the associate editor.

REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS.

The changes made at the 1927 meeting of the association were published in the February, 1928, number of *The Journal*. Thus the compilation of all changes made since the 1925 revision of *Methods of Analysis* is complete. The changes made at the 1924 meeting were not compiled in a similar form, but they may be found in the reports of subcommittees A, B, and C of the Committee on Recommendations of Referees, published in the February, 1925, number of *The Journal*. According to the order of publication followed at present, these reports as well as all other committee reports are given in the first number of each volume of *The Journal*.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS
OF ANALYSIS MADE AT THE FORTY-FOURTH ANNUAL
CONVENTION, OCTOBER 29-31, 1928¹.

I. FERTILIZERS.

(1) In the official gravimetric method for the determination of total phosphoric acid, the words "Nearly neutralize with strong hydrochloric acid" (p. 3, 7, line 12) were deleted, and the words "Neutralize with strong hydrochloric acid, using litmus paper or bromthymol blue as indicator" were substituted therefor (final action).

(2) In the official gravimetric method for the determination of total phosphoric acid, the words, "burn first at a low heat and then ignite intensely until white or grayish white" (p. 3, 7, line 17), were deleted, and the directions, "burn first at a low heat and ignite to constant weight, preferably in an electric furnace, at 950°-1000°C.", were substituted therefor (final action).

(3) A third alternative method for the preparation of magnesia mixture [p. 2, 5 (c)] reading as follows: "(3) Dissolve 55 grams of crystallized magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using, at the rate of 15 cc. per 100 cc. of solution", was adopted as official (final action).

(4) The official zinc-iron method for the determination of nitric and ammoniacal nitrogen (p. 11, 35), which was placed under the subheading "Nitrogen in Nitrate Salts" (p. 12) at the 1925 meeting, was dropped (final action).

(5) The use of the official reduced iron method for the determination of nitric and ammoniacal nitrogen (p. 11, 34) was restricted by inserting the sentence, "Applicable only in the absence of cyanamide and urea" (final action).

(6) The official method for the determination of water-insoluble organic nitrogen soluble in neutral permanganate (p. 12) was amended by inserting the word "water-insoluble" before the word "nitrogen" in line 3 of paragraph 38 (final action).

(7) In the official method for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 12) the directions in paragraph 40 for the preparation of the alkaline permanganate solution were changed to read as follows: "Dissolve 25 grams of potassium permanganate in hot water and, separately, 150 grams of sodium hydroxide in cold water; combine the solutions when cold and dilute to 1 liter. Discard any permanganate solutions that have become green in color" (final action).

¹ Unless otherwise stated, all references in this report are to *Methods of Analysis*, A. O. A. C., 1925.

(8) The directions for the preparation of sample for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 13) were amended by adding to paragraph 41 (a) the following: "When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed" (final action).

(9) The directions for the determination of water-insoluble organic nitrogen distilled from alkaline permanganate (p. 13, 42) were changed (final action) as published in *This Journal*, 1928, 11: 34.

(10) The method for the determination of chlorine in fertilizers adopted as tentative last year¹ was adopted as official (first action).

II. SOILS.²

(1) The heading for the tentative method for the determination of ferric and aluminum and titanium oxides and phosphorus (p. 28) was changed to the following: "Oxides of iron, aluminum, manganese, phosphorus, and titanium", and the coprecipitation of manganese was provided for by the following insertions and changes:

(a) Following the first sentence insert the sentence, "Add 0.5 gram of solid ammonium persulfate".

(b) Change the next to the last sentence, first paragraph, to read, "Reprecipitate the oxides with ammonium persulfate and dilute ammonium hydroxide as directed above, etc."

(c) In the second sentence, second paragraph, insert the words, "Manganomanganic oxide (Mn_3O_4)" between " (Al_2O_3) and titanium oxide".

(d) Before the last paragraph insert a paragraph to read as follows: "Evaporate 50-100 cc. of the solution from 17 after the addition of 10 cc. of concentrated nitric acid. Repeat the addition of nitric acid and evaporation to insure expulsion of all hydrochloric acid. From this point proceed as directed in 75, p. 101.

(e) Change the last paragraph to read as follows: "Subtract the sum of the oxides of iron, manganese, and phosphorus (determined separately as directed in 27 or 28) from the weight of the combined oxides of iron, aluminum, manganese, phosphorus, and titanium, determined as directed above. Report the remainder as oxides of aluminum and titanium".

III. AGRICULTURAL LIMING MATERIALS.

The sugar method for the determination of the caustic value of lime³ was adopted as an official method (final action).

¹ *This Journal*, 1928, 11: 34.

² Changes recommended by the Committee on Revision of Soil Analysis.

³ *This Journal*, 1928, 11: 152.

IV. PLANTS.

The following methods for the determination of copper, manganese, and zinc were adopted as tentative methods.

COPPER.

REAGENTS.

(a) *Potassium ethyl xanthate*.—0.1 per cent water solution prepared fresh each time it is used.

(b) *Standard copper sulfate*.—Dissolve 3.9283 grams of pure cupric sulfate in water and dilute in a volumetric flask to 1000 cc. and mix. Dilute 10 cc. of this stock solution in a graduate flask to 1000 cc. 1 cc. = 0.0001 gram Cu.

(c) *Filter-paper pulp*.—Moisten and tear a good grade of sheet filter paper into bits and place in a porcelain dish of the proper size. Add, while stirring with a glass rod, enough cold concentrated hydrochloric acid to disintegrate the paper and reduce the mass to a mushy consistency. Transfer the pulp to a large Büchner funnel and wash free of acid, using suction. Transfer the washed pulp into a clean glass bottle and add water to make a thick pulp suitable for making a pad in a Caldwell crucible. (The precipitates of copper and zinc obtained in these methods can be readily filtered and washed upon pads made with this filter-paper pulp by the use of the suction pump.)

(d) *Hydrochloric acid*.—0.25 *M* solution of C. P. hydrochloric acid.

DETERMINATION.

Ash 100–500 grams of the finely divided air-dried plant material in silica dishes with a small flame. Do not allow the plant material to burn with a blaze. After the volatile matter has been dispelled, complete the ashing in a muffle furnace maintained at the temperature of a faint red glow. Hasten the ashing process by removing the dishes from the muffle at intervals and breaking up the lumps with a platinum stirring rod. After the carbon has been oxidized as completely as possible cool the dish, moisten the ash with water, wash into a 250 cc. beaker, and cover with a watch glass. Decompose the ash with hydrochloric acid (1 + 1) introduced through the lip of the beaker beneath the watch glass by means of a pipet. After effervescence has ceased, rinse the watch glass into the beaker and filter the insoluble residue out on a Büchner funnel and wash free of chlorides. If the insoluble residue contains undecomposed particles of carbon transfer it into a silica dish and reignite in the muffle furnace until all particles of carbon are decomposed and a light colored ash remains. Redigest the ash on a hot water bath with 15 cc. of hydrochloric acid (1 + 1), filter, and wash free of chlorides. Combine the filtrates in a clean porcelain dish, evaporate to dryness, and bake at 110°C. until hydrochloric acid is expelled. Moisten the dry residue with 10 cc. of hydrochloric acid (1 + 1) and digest, with stirring, for 10 minutes. Dilute with hot water, filter out the silica, wash free of chlorides, combine with the insoluble residue, ignite, and weigh. Make the filtrate to about 250 cc., heat to near the boiling point, and pass a slow stream of hydrogen sulfide through the solution for 15 minutes. Rinse the hydrogen sulfide delivery tube into the flask, tightly stopper, and set aside until the precipitate settles and the supernatant solution is clear. Filter the copper sulfide off on a pad of paper pulp and wash with reagent (d) saturated with hydrogen sulfide, ignite in a porcelain crucible, and dissolve the copper oxide in a few drops of dilute nitric and one drop of dilute hydrochloric acid. Filter the solution and wash the filter paper clean. Evaporate the solution to dryness in a porcelain dish three times with the addition of a few drops of nitric acid and take up with a very small drop of dilute nitric acid delivered from a stirring rod having a sharp point. Make to a volume of 50 cc., and transfer an aliquot

of 5 cc. to a Nessler tube containing 10 cc. of reagent (a). Mix the solutions and dilute to 25 cc. Transfer 10 cc. of reagent (a) to a second Nessler tube, dilute to a volume of about 15 cc. and add the standard copper solution (b) dropwise with thorough mixing with a glass stirring rod until the color in the standard tube apparently matches the color in the tube containing the sample. Make the volume and the final adjustment of the color of the standard in the tube containing it. Record the number of cubic centimeters of the copper standard required and calculate the percentage of copper (Cu).

To the filtrate from the copper sulfide add 5 cc. of nitric acid (1 + 1) and boil for 10 minutes to oxidize the remaining metals to the ferric condition. Cool the solution and make to a convenient volume (250 cc.). From this stock solution take suitable aliquots for the determination of manganese, zinc, or other elements contained in the ash of the sample.

MANGANESE.

REAGENTS.

(a) *Potassium periodate pure salt*.¹

(b) *Standard manganous sulfate solution*.—Dissolve 0.2877 gram of pure potassium permanganate in about 100 cc. of water, acidify the solution with dilute sulfuric acid (1 + 1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10 per cent oxalic acid solution to discharge the color and boil for 5 minutes to expel the carbon dioxide; cool and dilute to 1 liter. 1 cc. of this solution = 0.1 mg. Mn.

DETERMINATION.

Transfer an aliquot of the stock solution equivalent to 5 grams of the air-dried plant material into a porcelain dish, add 20 cc. of sulfuric acid (1 + 1), and heat on the water bath until hydrochloric acid is completely expelled. Transfer the solution to a 150 cc. Erlenmeyer flask, add about 0.05 gram of reagent (a), and boil until the permanganic acid color has developed; continue to heat the Erlenmeyer flask on a hot water bath for 1 hour; cool, make to a volume of 100 cc., mix, and transfer an aliquot of 25 cc. to a 50 cc. Nessler tube. Match the sample with one of a series of manganese color standards made from reagent (b) and calculate the percentage of manganese (Mn) contained in the plant material.

ZINC.

REAGENTS.

(a) *Potassium ferrocyanide*.—2 per cent of freshly prepared solution.

(b) *Zinc sulfate*.—Dissolve 1 gram of C. P. zinc in sulfuric acid and dilute in a volumetric flask to 1000 cc. 1 cc. = 0.001 gram of zinc.

DETERMINATION.

Transfer an aliquot equivalent to 25 grams of plant material of the stock solution to a 250 cc. Erlenmeyer flask and add ammonia in slight excess. Dissolve the precipitate in a slight excess of pure acetic acid, saturate the solution with hydrogen sulfide and set aside for several hours for the precipitate to settle. Filter on a pad of paper pulp and wash with a dilute solution of acetic acid containing ammonium acetate saturated with hydrogen sulfide; ignite the pad of paper pulp and precipitate in a porcelain crucible; cool; dissolve the residue in a few drops of dilute hydrochloric acid and warm on the hot water bath; transfer to a 100 cc. beaker and add ammonia in slight excess; heat on the water bath for 5 minutes, filter, and wash the precipitate.

¹ A pure product can be obtained from Messrs. Eberbach & Son Co., Ann Arbor, Mich.

Add acetic acid in a slight excess to the filtrate and saturate the hot solution with hydrogen sulfide; stopper tightly and set aside several hours in a warm place for the precipitate to settle; filter; wash as previously described and ignite in a porcelain crucible. Dissolve the ignited residue of zinc oxide in 10 cc. of 0.1 *N* sulfuric acid, make to a volume of 50 cc., and mix. Transfer an aliquot of 5 cc. to a 50 cc. Nessler tube containing 5 cc. of reagent (a). Dilute to 50 cc. and mix with a stirring rod. To another Nessler tube containing 5 cc. of reagent (a) and diluted to about 40 cc. add dropwise with stirring the standard solution (b) until the turbidity in the standard matches the turbidity of the sample. From the number of cubic centimeters of the zinc standard required calculate the percentage of zinc (Zn) contained in the sample.

V. INSECTICIDES AND FUNGICIDES.

(1) The tentative method for the determination of copper in Bordeaux Paris green and Bordeaux calcium arsenate (p. 63), as revised by the referee, was adopted as an official method (first action). The revision is as follows: For the second sentence substitute the following sentence: "Add 25 cc. of hydrogen peroxide solution; dilute to about 100 cc.; and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2-3 amperes".

(2) The tentative method for the determination of unsulfonated residue in mineral oils¹, amended by striking out the third sentence, which reads "In lieu of measuring, determine the specific gravity of the oil and weigh the equivalent of 5 cc. into the bottle", and substituting therefor the following: "(If greater accuracy is desired, the measured charge may be weighed and its exact volume calculated from the weight and specific gravity of the oil)" was adopted as official (final action).

VI. TANNING MATERIALS.

No additions, deletions, or other changes.

VII. LEATHERS.

No additions, deletions, or other changes.

VIII. WATERS, BRINE, AND SALT.

No additions, deletions, or other changes.

IX. FEEDING STUFFS.

The toluene distillation method (Bidwell-Sterling²) for the determination of moisture was adopted as official (final action). First action was taken in 1926.

¹ *This Journal*, 1927, 10: 30.

² *Ibid.*, 1926, 9: 30.

X. PRESERVATIVES AND ARTIFICIAL SWEETENERS.

No additions, deletions, or other changes.

XI. COLORING MATTERS IN FOODS.

No additions, deletions, or other changes.

XII. METALS IN FOODS.

No additions, deletions, or other changes.

XIII. SUGARS AND SUGAR PRODUCTS.

(1) The directions for preparing the reagent, standard thiosulfate solution, in the official volumetric thiosulfate method (p. 191) were revised as follows (first action):

Standard thiosulfate solution.—Prepare a solution of sodium thiosulfate containing 19 grams of pure crystals in 1 liter. Weigh accurately about 0.02 gram of pure copper and place in a flask of 250 cc. capacity. Dissolve by warming with 5 cc. of a mixture of equal volumes of strong nitric acid and water. Dilute to 50 cc., boil to expel the red fumes, add a slight excess of strong bromine water, and boil until the bromine is completely driven off. Cool, and add a strong sodium hydroxide solution with agitation until a faint turbidity of cupric hydroxide appears. Discharge the turbidity with a few drops of 80 per cent acetic acid and add 2 drops in excess. (The solution should now occupy a volume of 50–70 cc.) Add 10 cc. of 30 per cent potassium iodide solution. Titrate at once with the thiosulfate solution until the brown tinge becomes weak and add sufficient starch indicator [p. 48, 3 (e)] to produce a marked blue coloration. Continue the titration cautiously until the color changes toward the end to a faint lilac. (If at this point the thiosulfate is added drop by drop and a little time is allowed for complete reaction after each addition, no difficulty is experienced in determining the end point within a single drop.) 1 cc. of the thiosulfate solution = about 0.005 gram of copper.

(2) The last two lines of the volumetric thiosulfate method, p. 192, 38, was revised as follows: under 37, beginning with “Cool and add strong sodium hydroxide solution” (first action).

(3) The first paragraph under “Determination”, Lane-Eynon general volumetric method¹ was revised as follows:

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 cc. of Soxhlet's solution 15 cc. of the sugar solution and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar solution until only the faintest perceptible blue color remains. Then add 2–5 drops of methylene blue and complete the titration by adding the sugar solution drop by drop. (The error resulting from this titration will not generally exceed 1 per cent.)

XIV. FRUITS AND FRUIT PRODUCTS.

No additions, deletions, or other changes.

¹ *This Journal*, 1926, 9: 35.

XV. CANNED VEGETABLES.

The following method for estimating field corn in canned mixtures of field and sweet corn was adopted as tentative:

Empty the contents of the can (No. 2), or the representative equivalent portion of a larger can, into a large beaker and remove the liquor and debris from fragments of kernels by flotation with cold water. Place upon a flat plate all kernels to which the outer seed coat is still attached, mix thoroughly, and quarter to about 400 pieces. Harden the selected pieces in 95 per cent alcohol and quarter again to obtain about 100 fragments. Cut each fragment through with a section razor or knife and avoid contamination of the fragments with dextrin by washing and drying the instrument after each cut. With a dissecting needle remove a portion about $\frac{1}{8}$ inch in diameter from the uncontaminated interior of each kernel and place the pieces in separate depressions of a white spot plate. Cover each piece with iodine stain (0.2 gram of iodine, 1.5 grams of potassium iodide in 100 ml. of water) and allow to stand 10 minutes. A dense brown cloud will disseminate from the portions of sweet corn due to dextrin, while the solution surrounding the field corn will remain clear and the portion will be blue black and sharply outlined. Crush the field corn portions to insure absence of dextrin and count those found to contain none. Calculate the percentage of field corn from the total number of kernels examined.

XVI. CEREAL FOODS.

FLOUR.

(1) The following method for the determination of glutenin was adopted as a tentative method:

GLUTENIN.

REAGENTS.

(a) *Barium hydroxide*.—Freshly powdered.

(b) *Methyl alcohol*.—96 per cent; free from acids, aldehydes, and ketones. Synthetic methanol preferred.

Flour and reagents should be allowed a minimum exposure to the air at all times.

DETERMINATION.

Weigh 8 grams of flour into a 200 cc. flask, preferably a sugar flask, or one that readily permits thorough mixing of the suspension when shaken. Add 0.2 gram of barium hydroxide, follow at once with 50 cc. of distilled water (carbon-dioxide free), and stopper tightly. Shake immediately to form a smooth suspension. Digest for 1 hour at room temperature, shaking frequently. Add sufficient methyl alcohol to allow 5 cc. of liquid above the mark (to correct for volume of flour) when thoroughly mixed. Shake vigorously for 2 minutes. After the starch settles to the bottom, pour the supernatant liquid *at once* through a cotton plug, repeating the filtrations two or three times if necessary. Immediately withdraw 50 cc. for the Kjeldahl nitrogen determination. Do not allow more than 15 minutes to elapse from the time the methyl alcohol is added to the withdrawal of the 50 cc. aliquot, because gliadin will begin to precipitate after standing for a short period of time. To prevent troublesome foaming add 150–200 cc. of water to the Kjeldahl flask before starting the digestion of the alcoholic extract. Convert the nitrogen to protein by the factor 5.7, subtract the percentage of protein

in the extract from the percentage of total protein ($N \times 5.7$) as determined in a separate portion of flour, and record the difference as the percentage of glutenin in the flour.

(2) The official method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol¹ was dropped, and the following slightly modified method was adopted as official (first action):

**WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT
ALCOHOL.—TENTATIVE.**

REAGENTS.

(a) *40 per cent alcohol*.—Mix 50 volumes of water and 35 volumes of 95 per cent alcohol.

(b) *Asbestos*.—Ignite and rub through an 8-mesh sieve.

DETERMINATION.

Place 20 grams of flour in an 8 ounce nursing bottle, add 100 cc. of water from a pipet, shake the bottle vigorously to prevent lumping of the sample, and add exactly 100 cc. more of water. Shake the stoppered bottle mechanically or by hand for 1 hour. (The temperature of the water should not exceed 30°C.)

Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. (The filtrate should be practically clear.) Pipet 50 cc. of the filtrate into an 8 ounce nursing bottle. Add 0.6 gram of sodium chloride and dissolve. Add 0.2 gram of ignited asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Allow to stand overnight, then centrifugalize to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off and wash with two 20 cc. portions of 40 per cent alcohol, in each case shaking, centrifugalizing, and decanting. If the liquid is not free of suspended matter, filter through a thin asbestos pad (0.1–0.15 gram) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos from the nursing bottle to a Kjeldahl flask with the aid of a stream of water, adding to it the mat in the Gooch crucible, and determine the nitrogen by the Kjeldahl-Gunning-Arnold method (p. 8, 22), using about 40 cc. of 0.1 *N* acid to receive the distillate. Make a blank determination on the reagents.

BAKED CEREAL PRODUCTS.

(1) The tentative method for the determination of total solids in an entire loaf of bread² was made official (first action).

(2) The official method for the determination of fat (by acid hydrolysis) in flour³ was adopted as a tentative method for baked cereal products.

(3) The official method for the determination of chlorides in the ash of alimentary paste (p. 232)⁴ was adopted as an official method for baked cereal products (first action).

(4) The official method for the determination of moisture in flour⁵ was

¹ *This Journal*, 1926, 9: 40.

² *Ibid.*, 42.

³ *Ibid.*, 41. See also, 1928, 11: 38.

⁴ *Ibid.*, 1927, 10: 34.

⁵ *Ibid.*, 1926, 9: 39.

adopted as an official method for air-dried baked cereal products (first action).

(5) The official method for the determination of crude fiber in flour (p. 225) was adopted as an official method for air-dried baked cereal products (first action).

(6) The official method for the determination of organic and ammoniacal nitrogen in alimentary paste (p. 232) was adopted as an official method for air-dried baked cereal products (first action).

(7) The following standard experimental baking test was adopted as tentative:

EXPERIMENTAL BAKING TEST.—TENTATIVE.

Basic Method.

EQUIPMENT.

Mixing bowl.—Ordinary graniteware "oatmeal bowl", top diameter 6½ inches, bottom diameter 2½ inches, and depth 2½ inches.

Fermentation bowl.—Same as mixing bowl, but smaller. Top diameter 5 inches, bottom diameter 2 inches, and depth 2½ inches.

Spatula.—Flexible steel blade, approximately 5 inches long, ¾ inch wide.

Baking pan.—Bottom inside, 53 x 93 mm.; height (sides), 85 mm.; height (ends), 68 mm.; top inside (at height of 68 mm.), 60 x 105 mm. Pans preferably of rust-proof material requiring little or no greasing.

Fermentation cabinet.—With precise temperature control.

Baking oven.—With precise temperature control.

INGREDIENTS.

Flour.—100 grams on a 15 per cent moisture basis (85 grams of dry matter).

Yeast.—3 grams (3 per cent).

Salt.—1 gram (1 per cent) 99.5 per cent pure.

Sugar (sucrose).—2.5 grams (2½ per cent).

Water (distilled).—Sufficient to make 58 per cent absorption with the flour on a 15 per cent moisture basis. Determine the exact quantity of water to be added by subtracting from 158 the weight of flour used as previously computed.

PROCEDURE.

Mixing.—Dissolve the salt and sugar in a portion of the water. Disintegrate the weighed portion of yeast in the salt-sugar solution or in another portion of the water. If the former procedure is followed, do not allow the yeast to remain in the salt-sugar solution for any considerable time before adding to the flour. If stock solutions are used, correct for the water added. Add the flour and mix with a flexible spatula which will conform readily to the shape of the bowl, making 125 cuts with the spatula. So regulate the temperatures of the ingredients that the dough comes from the mixing operation at 30°C. Remove the dough from the bowl and fold 20 times in the hands.

Fermentation.—Place the dough in a fermentation bowl and allow to ferment for 105 minutes at 30°C. (plus or minus 0.5°) and not less than 75 per cent relative humidity. Remove the dough from the bowl, fold 15 times in the hands (first punch), return the dough to the bowl, and allow fermentation to proceed as before for 50 minutes. Again remove the dough, fold 10 times (second punch), and replace in the fermentation bowl for 25 minutes. Remove the dough from the bowl, mold, and pan as follows:



Molding and panning.—Place the dough on a table or molding board and pound vigorously with the heel of the hand until the dough is flat and circular (Fig. 1). Holding one side of the dough, cut the mass loose from the table with the spatula, and turn on the reverse side. Fold over two opposite sides so that they overlap to a considerable degree (Fig. 2). Turn the dough over, and again pound it flat with the heel of the hand. Holding one end, cut the dough loose from the table with a spatula, and turn on reverse side with the seam of the dough running from the operator. Starting at the more remote end, roll the dough toward the operator, folding it as tightly as possible (Fig. 3). Seal the seam tightly, and with the seam underneath seal the ends by pinching them vertically. Roll lightly under the palm of the hand, adjusting the dough to the length of the pan, and place in the pan with the seam down. (The length of the dough should not exceed that of the pan prior to the final light rolling.) Use no dusting flour in the molding process.

Proof.—Proof 55 minutes under the same conditions as for fermentation.

Baking.—Bake 25 minutes at a temperature of 230°C. (plus or minus 2°) at the level of the top of the baking pan. (Precise control of temperature, both as to degree and uniformity, is essential.)

Measurement.—Weigh the loaf and measure its volume 30 minutes after removal from the oven.

Optional Variations.

1. Absorption:

Basic procedure in which absorption only is varied.

2. Fermentation:

Basic procedure varying fermentation time only.

3. Addition of special oxidizing reagent, Potassium Bromate:

Basic procedure with addition of potassium bromate in successive increments of 1 mg.

4. Mechanical modification:

Basic procedure varying mixing only.

ALIMENTARY PASTES.

(1) The official method for the determination of moisture in flour¹ was adopted as official for alimentary pastes (first action).

(2) The tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour² was modified by the associate referee and adopted as official for alimentary pastes (first action). (See p. 40, Recommendation 2.)

(3) The official method for the determination of crude fiber in flour (p. 225) was adopted as an official method for alimentary pastes (first action).

XVII. MEAT AND MEAT PRODUCTS.

(1) In the official method for the determination of total nitrogen in meat (p. 237, 6) the last sentence, "In the Kjeldahl and Gunning methods digest with sulfuric acid for at least 4 hours, in the Kjeldahl-Gunning-Arnold method, for 2 hours after the mixture has become clear", was deleted (final action).

(2) The following method for the detection of added water in sausage and similar meat products was adopted as tentative:

ADDED WATER IN SAUSAGE AND SIMILAR MEAT PRODUCTS.

PRELIMINARY PROCEDURE.

Place in a tared weighing bottle a sufficient quantity of the ground sample for both protein and moisture determinations, weigh accurately, and remove the portion for the protein determination. Weigh again, leaving the remainder in the weighing bottle for the determination of moisture and dry this portion in the weighing bottle.

DETERMINATIONS.

Water.—Weigh 5–7 grams of the sample into a tared dish or weighing bottle approximately 2 inches in diameter, spreading out the mixture in a thin layer over the sides and bottom of the dish with a small glass rod that was weighed with the dish and included in the tare.

Moisture.—Dry the sample in air at atmospheric pressure at a temperature of 101°–102°C. for approximately 16–18 hours, or until no significant loss of weight occurs on subsequent drying for a period of 2 hours. (If preferred, moisture may be determined according to paragraph 2, p. 237.)

Nitrogen.—Determine total nitrogen according to paragraph 17, 22, or 24, p. 6. Calculate the protein by multiplying the total nitrogen by the factor 6.25.

Added Water.—Multiply the percentage of protein calculated from the nitrogen determination by 4 and subtract the result from the percentage of moisture found. Report the difference, if any, as added water.

XVIII. GELATIN.

No additions, deletions, or other changes.

¹ *This Journal*, 1928, 9: 39.

² *Ibid.*, 40.

XIX. DAIRY PRODUCTS.

BUTTER.

The words "or mix" in the second line of the official directions for the preparation of sample (p. 276, 67) were deleted (final action).

CHEESE.

(1) The phrase "* * * then add 2.5 cc. of the permanganate solution * * *", 19th line in the tentative method for the quantitative determination of citric acid¹ was corrected to read "* * * add 25 cc. of the permanganate solution * * *".

(2) The official Schmidt-Bondzynski method for the determination of fat in cheese (p. 279) was changed by substituting for the phrase, "* * * 0.5 gram of sand to prevent bumping * * *", the phrase "* * * about 0.5 gram of sand, previously digested with concentrated hydrochloric acid * * *" (first action).

XX. FATS AND OILS.

(1) The tentative method for the determination of liquid and solid fatty acids (p. 292) was deleted.

(2) The lead-salt-ether method for the determination of saturated and unsaturated fatty acids was adopted as official (first action). The method is as follows:

LEAD-SALT-ETHER METHOD.

REAGENTS.

(a) *Lead acetate solution*.—1000 cc. of a 20 per cent solution.

(b) *Potassium hydroxide solution*.—(1) Dissolve 50 grams of potash in 50 cc. of water; (2) dissolve 30 grams of potash in 200 cc. of water.

(c) *Acetic acid solution*.—Dissolve 25 cc. of glacial acetic acid in 50 cc. of water.

(d) *Alcohol*.—95 per cent.

(e) *Ether*.—U. S. P. grade.

DETERMINATION.

Weigh accurately 10 or 20 grams of the sample into a 200 cc. Erlenmeyer flask. Add 30 cc. of alcohol and 8 cc. of the concentrated potassium hydroxide solution (b) (1). Mix thoroughly and heat on the steam bath for about 30 minutes. Add a slight excess of acetic acid, using phenolphthalein as an indicator, and then add a sufficient quantity of the dilute potassium hydroxide solution while rotating the flask to produce a distinct pink color. Heat to boiling in a liter flask a mixture of 60 cc. (120 cc. for 20 grams of sample) of lead acetate solution and 60 cc. of water. Add the neutralized soap solution cautiously to avoid any loss, rinsing the saponification flask with 5 cc. of alcohol, then with small volumes of hot water. Boil the mixture gently for about 5 minutes, shake thoroughly, and cool under running water, rotating the flask to cause all the precipitated lead soaps to adhere to the sides and bottom of the flask. When the mixture is cold

¹ *This Journal*, 1928, 11: 42.

pour off the aqueous solution into a large beaker in order to examine the solution for particles of lead soap. (Usually the solution is slightly turbid owing to some basic lead acetate, and no particles or globules of lead soap are seen.) Wash the flask and lead soap twice with cold water and allow the flask to drain for 10 minutes. Remove the last drops of water by means of a thin roll of filter paper held by forceps, being careful to press the paper only lightly against the precipitate. Add about 120 cc. of ether and shake by rotating the flask for about 5 minutes.

Connect the flask with a reflux condenser and boil the contents gently until the lead soap is completely disintegrated or dissolved. Remove the flask and rinse down the sides with sufficient ether to make the final volume about 150 cc. Invert a close fitting beaker over the neck of the flask and place in an icebox for at least 15 hours. Place a 7 cm. ordinary filter paper in a Büchner funnel of 7.5 cm. diameter, turn on full suction, and fit a hardened filter paper cut to 8 cm. in diameter as snugly to the sides of the funnel as possible. Decant the ether solution from the separated lead soaps, using only sufficient suction to draw the ether through the filter. (Too much suction causes the ether to evaporate so rapidly that the filter may become clogged with the separated unsaturated acids, lead soaps, or ice.)

Transfer the precipitate to the filter by rinsing the flask with small portions of ether. During filtration keep the funnel covered as much of the time as possible to prevent the evaporation of the ether. If at any time filtration proceeds so fast as to cause the mass of lead soap to crack, close the cracks by pressing with a small spoon or spatula; otherwise the precipitate cannot be properly washed. Rinse the spoon free from the precipitate and wash the precipitate from eight to ten times with the ether, finally allowing the suction to continue until the precipitate cracks into numerous pieces. Without delay, separate with a spoon as much of the precipitate as possible and transfer it without loss to a 500 cc. separatory funnel containing about 50 cc. of ether, washing off any precipitate adhering to the spoon and neck of the separatory funnel with ether. Transfer the filter paper to the liter flask. Shake the contents of the separatory funnel thoroughly to disintegrate the lumps of lead salt and allow to stand for about 20 minutes. Add 20 cc. of hydrochloric acid previously diluted with 10 cc. of water and shake thoroughly for 2 minutes to decompose all the lead soap. Add 5-10 cc. of hydrochloric acid (2 + 1) to the liter flask containing the filter paper, shake thoroughly to decompose any precipitate adhering to the flask, filter, and then wash into the separatory funnel with small alternate portions of ether and water until all the fatty acids and lead chloride are removed from the flask. Again shake the contents of the separatory funnel with a rotary motion and allow to stand for 10 minutes. Withdraw the lower aqueous solution slowly, taking precautions not to remove any emulsion or undecomposed lead soap. When lead soap is present (shown in the form of lumps that float on top of the aqueous solution) add 10 cc. of hydrochloric acid and shake again; then add about 20 cc. of water, shake, and allow the mixture to stand until the layers have separated. Withdraw the aqueous solution and wash the ether with successive 25 cc. portions of water until the washings are free from hydrochloric acid. Dehydrate the ether with about 2 grams of anhydrous sodium sulfate and transfer the solution to a weighed 300 cc. Erlenmeyer flask. Rinse the separatory funnel and sodium sulfate with several small portions of ether to remove all the fatty acids, taking care not to allow any of the sodium sulfate to fall into the weighed flask. Distil the ether, avoiding any loss of the fatty acids, and heat in an oven at about 110°C. until the weight is constant. Obtain the weight of the saturated acids and save them for later investigation.

Transfer the ether solution of the soluble lead soaps to a 500 cc. or a 1000 cc. separatory funnel, rinsing the Büchner filter flask with small quantities of ether. Add a mixture of 30 cc. of hydrochloric acid and 75 cc. of water and shake with a rotary motion for 2 minutes. After allowing the mixture to stand for 10 minutes, slowly withdraw the aqueous solution into a beaker. If drops of the ether solution are entrapped by

the lead chloride precipitate and are removed with it, decant the solution from the precipitated lead chloride, which has settled, into the separatory funnel. Rinse the beaker and precipitate with small quantities of ether, adding the washings to the separatory funnel. Rotate the contents of the separatory funnel and allow to stand for 10 minutes. Withdraw the aqueous solution and wash the ether with successive 50 cc. portions of water until the hydrochloric acid is removed. Transfer the ether solution to a 300 cc. weighed Erlenmeyer flask. Distil the ether and place the flask in an oven heated to about 110°C. for about 1 hour, while passing a stream of carbon dioxide into the flask to prevent oxidation of the unsaturated acids. Cool in an atmosphere of carbon dioxide. When cold, remove the carbon dioxide and weigh. Repeat this treatment until a constant weight is obtained.

Determine in duplicate the iodine numbers of both the saturated and unsaturated acid fractions. (The iodine number of the saturated acid fraction is due to the presence of some unsaturated acid.)

To correct for the unsaturated acids present in the fraction of saturated acids use the following formula:

$$\frac{\text{Iodine No. of saturated acid fraction}}{\text{Iodine No. of unsaturated acid fraction}} \times 100 = A \quad \left(\begin{array}{l} \text{percentage of unsaturated acid in} \\ \text{saturated acid fraction} \end{array} \right).$$

Obtain the correct value by means of the formula $\frac{A \times B}{100}$, in which B is the percentage of the impure saturated acids (as found by analysis). Subtract this corrected value from the percentage of impure saturated acids and add it to the percentage of unsaturated acids actually determined.

(3) The cold test for testing salad oils, other than olive, was adopted as tentative. The method follows:

COLD TEST.

(Applicable to all salad oils except olive oil.)

Fill a 4 ounce sample bottle with the oil at a temperature of 25°C., insert the cork stopper tightly, and seal with paraffin. Submerge the bottle completely in a bucket containing finely cracked ice and add water until it rises to the top of the bottle. Keep the bucket filled solidly with the cracked ice by removing any excess water and adding ice when necessary. At the end of 5 hours remove the bottle and examine the oil. If it is properly wintered, the sample will be brilliant, clear, and limpid.

XXI. BAKING POWDERS AND BAKING CHEMICALS.

The following method for the determination of aluminum by precipitation with phenylhydrazine was adopted as tentative:

ALUMINUM.

REAGENTS.

(a) *Ammonium bisulfite solution*.—Pass sulfur dioxide into a cool, dilute solution of ammonia (1 + 1) until the color of the solution becomes distinctly yellow.

(b) *Phenylhydrazine bisulfite solution*.—To a few cubic centimeters of phenylhydrazine add gradually a saturated solution of sulfur dioxide until the precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved.

If the precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate of phenylhydrazine is obtained. Filter the solution before using. (From 5–10 cc. of this solution in 100 cc. of water is sufficient strength for washing the alumina precipitate. This concentrated solution of phenylhydrazine bisulfite, if well stoppered, will keep indefinitely.)

(C) *Dilute hydrochloric acid.*—Add 10 volumes of water to 4 of strong hydrochloric acid.

DETERMINATION.

Ignite 3 grams of baking powder at a temperature not exceeding 550°C. As soon as the carbon has burned off, take up the residue in hydrochloric acid (4 + 10) and boil gently to assist solution. Filter into a 300 cc. volumetric flask and wash with hot water. Ignite the insoluble residue and filter paper in a platinum crucible, and then fuse the residue with about 2 grams of sodium carbonate. Dissolve the fused mass in water and hydrochloric acid and transfer to the volumetric flask containing the original filtrate. Cool, and make up to volume.

Transfer 100 cc. aliquots to 400 cc. beakers. Heat nearly to boiling, add dilute ammonium hydroxide until a slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of dilute hydrochloric acid. Add, drop by drop with constant stirring, 10 or 12 drops of a saturated solution of ammonium bisulfite. Then add to the hot solution sufficient phenylhydrazine to precipitate the alumina completely. (1 or 2 cc. is generally enough; an excess colors the solution yellow.) If a permanent precipitate does not form at this point, add dilute ammonia carefully, drop by drop, just to a permanent precipitate, and then complete the precipitation by adding a few more drops of the phenylhydrazine. Let stand a few minutes for the precipitate to settle, then filter while still warm. Wash the precipitate with warm water containing the phenylhydrazine bisulfite until the washings give no test for iron when yellow ammonium sulfite is added.

Place the filter paper containing the precipitate in a weighed platinum crucible. Dry, char, and ignite at a low temperature. After the filter paper has completely burned, continue the ignition at a bright red heat to constant weight. Weigh quickly with cover on the crucible as the precipitate is very hygroscopic. A second weighing is always necessary. The precipitate consists of aluminum oxide and aluminum phosphate.

Fuse the ignited precipitate with about 2 grams of sodium carbonate and dissolve the fusion in dilute nitric acid (1 + 9). Transfer to a 250 cc. beaker and boil to insure that all the phosphoric acid is in the ortho state. Cool. Transfer to a 200 cc. flask, make up to volume, and use 50 cc. aliquots to determine the phosphoric anhydride. Multiply the weight of phosphoric anhydride obtained by 4 and subtract the product from the weight of combined precipitates obtained above. The difference is the weight of aluminum oxide in 1 gram of baking powder.

$\text{Weight Al}_2\text{O}_3 \times 100 = \text{percentage of Al}_2\text{O}_3.$

$\text{Percentage of Al}_2\text{O}_3 \times 4.749 = \text{percentage of Na}_2\text{Al}_2(\text{SO}_4)_4.$

If the baking powder contains a significant quantity of silica, remove it by evaporating the hydrochloric acid solution of the powder to dryness and dehydrating at 105°C. for 2 hours. Add to the dry mass 10 cc. of hydrochloric acid and 100 cc. of water, boil, filter off the silica, and proceed as in the method described above.

XXII. SPICES AND OTHER CONDIMENTS.

No additions, deletions, or other changes.

XXIII. VINEGARS.

No additions, deletions, or other changes.

XXIV. COFFEES.

No additions, deletions, or other changes.

XXV. TEAS.

No additions, deletions, or other changes.

XXVI. CACAO PRODUCTS.

No additions, deletions, or other changes.

XXVII. FLAVORING EXTRACTS.

(1) The steam distillation method for the determination of oils of lemon, orange, and limes in corn and cottonseed oils and in mineral oil¹ was adopted as an official method (final action).

(2) The colorimetric method for the determination of small quantities of anthranilic acid ester² was adopted as an official method (final action).

(3) The gravimetric method for the determination of large quantities of anthranilic acid ester² was adopted as an official method (final action).

(4) The official method for citral in lemon oil and/or extracts³ was dropped (first action).

(5) The following method for the determination of citral in lemon oil and/or extracts was adopted as an official method to replace the present official method (first action):

CITRAL.

(Lemon and orange extracts.)

REAGENTS.

(a) *Metaphenylenediamine hydrochloride-oxalic acid solution*.—Dissolve 1 gram of metaphenylenediamine hydrochloride in about 45 cc. of 85 per cent alcohol and 1 gram of crystallized oxalic acid in a similar quantity of alcohol of the same strength and pour the two solutions into a 100 cc. volumetric flask. Add 2 or 3 grams of fullers' earth, dilute to the mark with 85 per cent alcohol, mix, and filter through a double-folded filter.

(b) *Alcohol*.—95 per cent by volume for lemon and orange extracts; 50–95 per cent by volume for terpeneless lemon and orange extracts.

(c) *Standard citral solution*.—Dissolve 1 gram of citral in alcohol, 90–95 per cent by volume, dilute to 100 cc., and mix. Dilute 5 cc. of this solution to 50 cc. with alcohol, 90–95 per cent by volume, and mix. 1 cc. of the second solution = 1 mg. of citral.

¹ *This Journal*, 1928, 11: 45.

² *Ibid.*, 46.

³ *Methods of Analysis*, A. O. A. C., 1925, 354.

DETERMINATION.

Weigh 25 grams of the extract into a 50 cc. volumetric flask, dilute to the mark with alcohol, and mix. Pipet 2 cc. or other suitable quantity of this solution into a colorimeter tube, add 10 cc. of the metaphenylenediamine hydrochloride-oxalic acid solution, dilute to suitable volume, and compare the resulting color with the colors of a set of standards containing known quantities of citral prepared like the sample.

XXVIII. WINES.

No additions, deletions, or other changes.

XXIX. DISTILLED LIQUORS.

No additions, deletions, or other changes.

XXX. BEERS.

No additions, deletions, or other changes.

XXXI. DRUGS.

(1) The following method for the determination of methyl and ethyl alcohols in the presence of each other, when methyl alcohol is present in small quantities (5 per cent or less), was adopted as a tentative method:

DETERMINATION OF ETHYL ALCOHOL IN THE PRESENCE OF METHYL ALCOHOL.

(When methyl alcohol is present in small amounts, 5 per cent or less.)

PREPARATION OF STOCK SOLUTION OF METHYL ALCOHOL.

Solution A.—Prepare a solution of methyl alcohol, adjusting its strength to 25 per cent by volume (± 0.1 per cent), using the specific gravity tables given in Allen's Commercial Organic Analysis, 5th ed., vol. 1, p. 89, or Van Nostrand's Chemical Annual, 5th ed., p. 591.

Solution B.—Prepare a standard solution of methyl alcohol by taking 20 cc. of Solution A and 95 cc. of absolute alcohol (or the equivalent of this amount in dilute alcohol) and making up to a volume of 2 liters. Make all transfers and dilutions at 20°C.

DETERMINATION.

Total alcohols.—Measure at room temperature (20°C.) 25 cc. of sample, add 90 cc. of water, neutralize to litmus with dilute sodium hydroxide, distil, and dilute the volume of distillate to 100 cc. at the same temperature as noted when the original aliquot was measured. Determine the total alcohol (as ethyl alcohol) from the specific gravity of the distillate in the usual way and estimate the percentage of alcohol in the original solution by means of the proper dilution factor. Test a portion of this distillate by the regular U. S. P. test for methyl alcohol, taking precaution to determine that formaldehyde, as such, is not present. If methyl alcohol is present, transfer 10 cc. of the distillate to a separatory funnel, add 40 cc. of saturated salt solution, shake with 25 cc. of petroleum ether, and draw off the aqueous salt solution into a distilling flask. Wash the petroleum ether in the separatory funnel with two 10 cc. portions of saturated

salt solution, adding these to the portion already in the distilling flask. Distil, receiving the distillate in a 50 cc. graduated flask. Calculate the amount of ethyl alcohol which it will be necessary to add to this distillate to make a 5 per cent solution of total alcohol (assuming it to be all ethyl alcohol) when made up to 50 cc., add this calculated amount, and make up to a volume of 50 cc. Transfer 5 cc. of this distillate to a 200 cc. flask for color comparison with standards.

Color standards.—Transfer to 200 cc. flasks a series of aliquots, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 cc. of Solution B, adding 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, and 0 cc., respectively, of 5 per cent ethyl alcohol. (These amounts of methyl alcohol represent percentages in the original unknown solution when the unknown is diluted as outlined under "Determination".)

Methyl alcohol (substantially as given in the U. S. P.).—To each of the standards and to the unknown, add 1 cc. of dilute phosphoric acid (1 + 1) and 2 cc. of 3 per cent potassium permanganate solution and allow the mixtures to stand for 10 minutes. Add 1 cc. of 10 per cent oxalic acid solution and allow the mixtures to stand until they have become clear or transparent. Add 5 cc. of dilute sulfuric acid solution (1 + 3) and 5 cc. of freshly prepared fuchsin-sulfurous acid mixture and allow the solution to stand for 1½ hours. Dilute to 200 cc., mix thoroughly, and transfer equal quantities to a series of test tubes of uniform color and diameter for color comparison. Compare the unknown with the standard which it most nearly approaches in color intensity, approximating intervals less than 0.5 per cent, if desired. The value obtained represents the percentage of methyl alcohol in the original sample.

Ethyl alcohol.—Deduct the percentage of methyl alcohol, as determined colorimetrically, from the percentage of total alcohols, as previously determined, to obtain the percentage of ethyl alcohol.

(2) The tentative method for the determination of arsenic in sodium cacodylate¹ was adopted as an official method (final action).

(3) The present tentative method for the determination of chloroform and carbon tetrachloride² was superseded by the following method, adopted as tentative:

CHLOROFORM AND CARBON TETRACHLORIDE.

REAGENTS.

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide in 30 cc. of water. Cool, and dilute to 100 cc. with methyl alcohol.

(b) *Alcoholic potassium hydroxide.*—Dissolve 35 grams of potassium hydroxide in sufficient methyl alcohol to make 100 cc. (This is a saturated solution.)

(c) *Silver nitrate.*—0.1 N solution.

(d) *Ammonium or potassium thiocyanate.*—0.05 N solution. Adjust by titrating against 0.1 N silver nitrate solution.

(e) *Nitric acid.*

(f) *Ferric ammonium sulfate indicator.*—Dissolve 8 grams of ferric ammonium sulfate in sufficient water to make 100 cc.

WEIGHING OF SAMPLE.

(1) *Chloroform or Carbon Tetrachloride:*

Carefully transfer 30 cc. of alcoholic potassium hydroxide solution into an air-dried, 60–70 cc. pressure bottle and stopper. Do not moisten the neck of the bottle with the reagent. [Reagent (a) is used for chloroform and Reagent (b) for carbon tetrachloride

¹ *This Journal*, 1928, 11: 48.

² *Ibid.*, 1927, 10: 45.

and for mixtures of chloroform or carbon tetrachloride.] Weigh the stoppered bottle with the contents. (The weighing may be conveniently made by suspending the bottle on the balance by means of the clamp that holds the stopper.)

Open the bottle and, in the case of chloroform or carbon tetrachloride, add about 1 cc. of the sample from a 1 cc. pipet, holding the pipet just above the top level of the reagent in the pressure bottle. As the level of the reagent rises with the draining of the sample into the bottle, raise the pipet correspondingly so as to avoid contact with the reagent. Avoid having the bottle open longer than necessary, 20 seconds being a convenient time. Stopper the bottle so as to assure a tight fit and weigh. Determine the weight by difference. Proceed as under "Determination".

(2) *Carbon Tetrachloride in Capsules:*

Ascertain the gross weight of a representative number of capsules. Open the capsules and transfer the contents to a suitable flask. Weigh the dried empty capsules and determine the average net contents. Proceed as under "Weighing of Sample", Part 1, using the composite sample.

(3) *Chloroform or Carbon Tetrachloride Mixtures:*

Proceed as under "Weighing of Sample", Part 1, using not more than 10 cc. of the mixture containing 0.08–1.6 grams of chloroform or carbon tetrachloride. Note the temperature of the mixtures. Ascertain the volume-equivalent of the weighed sample. Weigh a definite volume of the mixture at the same temperature, using a 50 or 100 cc. volumetric flask, and calculate.

NOTE.—If desired, the sample may be measured directly with a pipet instead of being weighed, or a measured volume may be diluted with methyl alcohol to some definite volume and thoroughly mixed and a suitable aliquot of this dilution used for the determination.

DETERMINATION.

If the sample is a mixture, mix the contents of the bottle by gentle swirling, and allow the bottle to stand about an hour (30 minutes is sufficient for chloroform, pure or nearly so). Then place the bottle in a wire basket and set the basket in a water bath at room temperature. Invert a tin can over the bottle and cover with a towel to prevent injury to the analyst in case the bottle should burst. Heat the bath to boiling and maintain at this temperature for 1 hour. (15 minutes is sufficient for chloroform, pure or nearly so.)

Cool gradually, transfer the contents of the pressure bottle to a 200 cc. volumetric flask, and wash out the bottle thoroughly with water, draining the washings into the flask. Bring to room temperature, fill to mark with water, and mix.

Transfer a suitable aliquot to a 100 cc. volumetric flask and acidify with nitric acid, adding about 2 cc. in excess. Add 25 or 50 cc. of 0.1 *N* silver nitrate (an excess), shake thoroughly, fill to mark with water, and mix. Filter the mixture through a dry filter into a dry flask, rejecting the first 20 cc. of the filtrate. To a 50 cc. aliquot of the filtrate, add 3 cc. of ferric ammonium sulfate indicator and titrate the excess 0.1 *N* silver nitrate, using 0.05 *N* ammonium or potassium thiocyanate.

If the original sample contains sugar or other organic material and after the saponification of the chloroform or carbon tetrachloride and dilution of the mixture with water is highly colored, thus interfering with the titration, transfer the contents of the pressure bottle to a nickel crucible with the aid of water. Evaporate to dryness and char the residue. Allow to cool, treat with water, and filter into a suitable volumetric flask, washing the residue and filter with water until free from chloride. Fill to mark with water and mix. Determine the chloride as directed in the preceding paragraph.

Make a blank test, using in the pressure bottle the same quantities of solvents and reagents as when the sample is present and apply whatever correction may be necessary

If the original sample contains chloride, which is precipitated by silver nitrate, determine the quantity and make correction.

1 cc. of 0.1 *N* silver nitrate = 0.003979 gram of chloroform or 0.003846 gram of carbon tetrachloride.

(4) The present tentative method for the determination of calomel in tablets¹ was amended to permit the use of asbestos as a substitute for paper in filtering as follows: For the directions "filter through a small filter paper. Wash well with water and return the paper and insoluble material upon it to the flask", substitute the following: "decant with the aid of suction through a tightly packed asbestos mat placed on the removable perforated plate of a Caldwell crucible. Wash once with water by decantation, then successively with alcohol and ether. Transfer the removable plate holding the asbestos mat and insoluble mat to the original flask, washing into the flask any insoluble material adhering to the sides of the crucible".

(5) The following method for the determination of mercurous iodide was adopted as a tentative method:

MERCUROUS IODIDE.

REAGENTS.

(a) *Purified iodine*.—Prepare as directed on p. 379, 2 (a).

(b) *Standard sodium thiosulfate solution*.—Prepare as directed on p. 379, 2 (b).

(c) *Standard iodine solution*.—Dissolve about 14 grams of iodine in a solution containing 18 grams of potassium iodide in 100 cc. of water and dilute to 1 liter. Standardize this solution against the standard sodium thiosulfate solution.

DETERMINATION.

Count and weigh a representative number of tablets. Pulverize a quantity of tablets and weigh accurately a sufficient portion of the well-mixed sample to represent 3–4 grains of mercurous iodide. Transfer the sample to a 200 cc. glass-stoppered Erlenmeyer flask, add about 50 cc. of water, and acidify with acetic acid. After the soluble fillers have dissolved, decant with the aid of suction through a tightly packed asbestos mat placed on the removable perforated plate of a Caldwell crucible. Wash once with water by decantation, then successively with alcohol and ether. Transfer the removable plate holding the mat and insoluble material to the original flask, washing into the flask any insoluble materials adhering to the sides of the crucible. Add 2.5 grams of potassium iodide and 30 cc. of the standard iodine solution. Allow the mixture to stand with frequent and fairly vigorous agitation for about 1½ hours, or until solution of the mercurous iodide is complete. Titrate with standard thiosulfate, adding 1 or 2 cc. in excess. When all traces of iodine have disappeared, titrate back with standard iodine, using starch indicator.

1 cc. of 0.1 *N* iodine solution = 0.03275 gram of mercurous iodide.

NOTE.—Some commercial tablets are difficult to filter without loss of mercurous iodide through the asbestos mat. A few drops of alumina cream, washed free from ammonia, placed on the mat before filtration is started, satisfactorily prevents loss though it retards the filtration.

¹ *This Journal*, 1928, 11: 51.

(6) The following microchemical tests for the identification of quinine, quinidine, cinchonine, and cinchonidine, were adopted as tentative:

MICROCHEMICAL TESTS FOR QUININE, QUINIDINE, CINCHONINE, AND CINCHONIDINE.

REAGENTS.

Five per cent solutions of each of the following: disodium phosphate, potassium iodide, sodium benzoate, platinic chloride, and sodium carbonate.

PREPARATION OF SAMPLES.

(1) *Controls*: Dissolve 1 mg. of pure alkaloidal salt in 2 drops of water to make an approximately 1-100 solution.

(2) *Alkaloids in compounds*: Separate the alkaloid in pure form by extracting it from an ammoniacal solution with a suitable immiscible solvent and evaporate the solvent. To 1 mg. of the residue add, drop by drop, 0.1 *N* hydrochloric acid, avoiding an excess of acid, and dilute with water, if necessary, to approximately the same alkaloidal concentration as in (1).

(3) *Hypodermic tablets*: Dissolve a portion of a tablet in water and dilute with water to approximately the same alkaloidal concentration as in (1).

Cinchona Alkaloids.

ALKALOID	REAGENT	CHARACTER OF PRECIPITATE
Quinine	Sodium phosphate	Silvery, sheaflike crystals.
Quinidine	Potassium iodide	Small triangular crystals in great numbers. Best in 1-1000 dilution. Crystals soluble when excess reagent is added.
Cinchonine	Sodium carbonate, sodium phosphate	Dark rosettes composed of radiating needles, forming immediately. Similar to crystals formed by sodium carbonate, but more burr shaped.
Cinchonidine	Sodium benzoate, platinic chloride, sodium carbonate	Rosettes and sheaves of needles spreading to large size. Rosettes of transparent plates. Spherical crystals, but not needles as in cinchonine.

Mixtures of Cinchona Alkaloids.

ALKALOID	SPECIFIC ROTATION IN ABSOLUTE ALCOHOL	SOLUBILITY IN ETHER	THALLEIOQUIN TEST*
Quinine	laevo 168°	1 gram in 1.8 cc.	Positive
Quinidine	dextro 256°	1 gram in 67 cc.	Positive
Cinchonine	dextro 225°	526 parts	Negative
Cinchonidine	laevo 108°	188 parts	Negative

* *Thalleioquin Test*: Add 1 or 2 drops of bromine water to 5 cc. of an aqueous solution of the alkaloidal salt (1 in 1000) and 1 cc. of ammonia (10 per cent). The liquid acquires an emerald-green color due to the formation of thalleioquin (U. S. P. X. p. 311).

IDENTIFICATION.

Place a drop of the alkaloidal solution on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the microscope, using low power. (A magnification of 100–150 is suitable.) Note the kind of crystals formed and compare their characteristics with the descriptions given and also with a control.

(7) The microchemical tests for the identification of atropine and pilocarpine¹ submitted in 1927 were adopted as tentative.

(8) The following method for the determination of pilocarpine in which methyl red is used as an indicator was adopted as a tentative method:

PILOCARPINE.

Ascertain the average weight per tablet. Pulverize, mix thoroughly, and weigh out a sufficient portion to represent 1 grain of the salt. Dissolve the sample in 10 cc. of water, add 1 cc. of dilute ammonium hydroxide, and shake out rapidly with 20 cc. of chloroform. Repeat the extraction, using 15 cc. of chloroform, and complete with successive 10 cc. portions. Filter each portion of the chloroform drawn off through a pledget of cotton and combine in a 250 cc. beaker, finally washing the stem of the separator and funnel with chloroform. Evaporate on the steam bath until the chloroformic solution measures about 5 cc. Add 20 cc. of 0.02 *N* sulfuric acid and evaporate the remainder of the chloroform. Titrate the excess acid with 0.02 *N* sodium hydroxide, using 1 drop of methyl red as indicator. (The end point is not particularly sharp, but with care it can be obtained.)

1 cc. of 0.02 *N* H_2SO_4 = 0.004893 gram of $\text{C}_{11}\text{H}_{17}\text{O}_2\text{N}_3 \cdot \text{HCl}$.

(9) The following method for the determination of thymol was adopted as a tentative method:

THYMOL.

Quantitative Method.

REAGENTS.

(a) *0.1 N bromine solution.*—Dissolve 3 grams of potassium bromate and 50 grams of potassium bromide in water. Dilute to 1 liter at 20°C. 1 cc. of 0.1 *N* solution = 0.003753 gram of thymol.

(b) *Hydrochloric acid solution* (1 + 1).

(c) *Methyl orange solution.*—0.1 gram to 100 cc.

(d) *Sodium hydroxide solution.*—25 per cent.

(e) *Potassium iodide.*

(f) *0.1 N sodium thiosulfate solution.*

(g) *Starch indicator.*—[p. 48, 3 (e)].

Determine the relationship between the bromine and thiosulfate solutions as follows: Transfer 25 cc. of the bromine solution to a 250 cc. glass-stoppered Erlenmeyer flask and add 25 cc. of water, a slight excess of hydrochloric acid, and 1 gram of potassium iodide. Titrate the liberated iodine with the thiosulfate solution, using starch solution as an indicator.

¹ *This Journal*, 1928, 11: 354.

DETERMINATION.

Weigh 2 grams of pulverized thymol, transfer to a 500 cc. volumetric flask, and add 25 cc. of the sodium hydroxide solution. Agitate until the thymol is dissolved and dilute to mark at 20°C., with water.

Method I: Transfer a 25 cc. aliquot of the thymol solution to a 250 cc. glass-stoppered Erlenmeyer flask, add 20 cc. of hot hydrochloric acid (1 + 1), and immediately run in 1–3 cc. less than the theoretical amount of bromine solution. Warm to 70°–80°C., add 2 drops of methyl orange solution, and titrate slowly with the bromine solution, swirling vigorously after each addition. When the red color of the methyl orange is bleached, add 2 drops of the titrating solution, stopper, shake vigorously for 10 seconds, add a drop of methyl orange solution, and again shake vigorously for 10 seconds. Continue the addition of 2 drops of bromine solution, shaking until the red color disappears. Then add 1 drop of methyl orange solution, shake vigorously, and if the red color does not disappear, repeat the alternate addition of 2 drops of bromine solution and 1 drop of methyl orange solution, shaking after each addition as directed above, until the red color disappears. Calculate the number of cubic centimeters of bromine solution used to percentage of thymol. Reserve the mixture in the titrating flask for Method II.

Method II: To the cooled mixture resulting from the titration according to Method I, add 3–5 cc. additional bromine solution. Stopper, shake, add 1 gram of solid potassium iodide, wash sides of flask and stopper with distilled water, and titrate the iodine liberated by the excess bromine solution with the standard thiosulfate solution, using starch solution as indicator. Calculate the amount of thiosulfate used in terms of bromine solution, deduct from the total amount of bromine solution added, and calculate to percentage of thymol.

To determine the approximate number of cubic centimeters of bromine solution required for Method I, heat a mixture of a 25 cc. aliquot of the sample and 20 cc. of hydrochloric acid (1 + 1) to about 80°C. and titrate slowly with the bromine solution, swirling vigorously while titrating until a yellow color, permanent for 1 minute, appears.

EGGS AND EGG PRODUCTS.

(1) The tentative method for the determination of the acidity of fat¹ was adopted as an official method (final action).

(2) The following method for the determination of ash was adopted as a tentative method:

ASH.

Weigh out 2–4 grams of liquid egg or 1 gram of powdered egg into an ashing dish, preferably a flat-bottomed platinum dish 5 cm. in diameter. (A porcelain dish of the same shape and capacity or a porcelain crucible of 20–25 cc. capacity may be used. Do not use silica dishes.) Add about 5 grams, accurately weighed, of previously ignited 60-mesh alundum (crystallized alumina), mix well, and then add from a pipet 5 cc. of an aqueous solution containing 1.5 grams of magnesium acetate per 100 cc. Run a blank determination on the magnesium acetate solution. Place the dish on an asbestos-centered gauze and evaporate carefully over a small Bunsen flame. Ignite carefully without removing the gauze and then heat the dish in a muffle at a low red heat for 2 hours. Cool the dish, weigh, break up the ash with a stirring rod, moisten with 5 cc. of water, evaporate to dryness on the steam bath, and heat in the muffle for an hour. Repeat until the dish ceases to lose weight. Subtract the weight of alundum added and

¹ *This Journal*, 1927, 10: 50.

the blank on 5 cc. of magnesium acetate solution from the weight of the residue to obtain the weight of egg ash.

(3) The following method for the determination of unsaponifiable matter was adopted as a tentative method:

UNSAPONIFIABLE MATTER.

Extract the lipoids according to the directions given previously¹. Determine the unsaponifiable matter in the extracted lipoids by the official F. A. C. method².

(4) The following method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in dried and liquid eggs was adopted as a tentative method:

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

PREPARATION OF SAMPLE.

(a) *Powdered egg*.—Place approximately 2 grams of the sample in an 8 ounce nursing bottle, add 25 cc. of ethyl ether, stopper with a water-soaked cork, shake for several minutes, centrifugalize until the supernatant liquid is clear, and carefully decant off the ether solution, allowing none of the egg to be carried along. Extract three more times with 20 cc. portions of ether in the same manner. Wash off the neck of the bottle carefully with ether after each decantation to remove adhering fat. Dry the fat-free residue by aid of suction and reduce to a fine powder by working with a glass rod. Add slowly 100 cc. of water from a pipet, shake vigorously to avoid lumping, and add exactly 100 cc. more of water. Shake the stoppered bottle mechanically or by hand for 1 hour. The temperature of the water should not exceed 30°C. Proceed as directed under "Determination".

(b) *Liquid egg*.—Weigh by difference approximately 10 grams of the well-mixed sample into an 8 ounce nursing bottle, add 100 cc. of dry ether, stopper with a water-soaked cork, and shake the mixture well. Centrifugalize lightly and carefully decant off the ether solution, allowing no solids to be carried over. Add 50 cc. of ether twice more, and shake, centrifugalize, and filter each time. Dry the fat-free residue by aid of suction. Add slowly 100 cc. of water, shake vigorously, and add exactly 100 cc. more of water. Shake the stoppered bottle mechanically, or by hand for 1 hour. Proceed as directed under "Determination".

DETERMINATION.

Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. (The filtrate should be practically clear.) Pipet off 50 cc. of the filtrate into a 200 cc. nursing bottle. Add 0.6 gram of sodium chloride and dissolve. Add 0.2 gram of ignited asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Let stand overnight and then centrifugalize to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off and wash with two 20 cc. portions of 40 per cent alcohol, in each case shaking, centrifugalizing, and decanting. If the liquid is not free of suspended matter, filter through a thin asbestos pad (0.1 — 0.15 gram) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos in the nursing bottle to a Kjeldahl flask with the aid of a stream of water, add to it the mat in the Gooch crucible, and determine the nitrogen by the official method (p. 8, 22), using about 40 cc. of 0.1 *N* acid to receive the distillate. Make a blank determination on the reagents and the asbestos.

¹ *This Journal*, 1925, 8: 602.

² *Ibid.*, 1926, 9: 45.

REPORT OF BOARD OF EDITORS.

The chairman of the Board of Editors gratefully acknowledges the assistance rendered by Dr. F. C. Blanck, who accepted the acting chairmanship for the greater part of the past year, during which time the present incumbent was unable to "carry on". To Miss Marian E. Lapp, associate editor, and to her assistant, Mrs. E. M. McCoy, he is deeply indebted for the efficient conduct of the many details of the arduous duties of preparing and editing the copy for *The Journal* and in caring for the many duties of the office for the year. He wishes to take this opportunity of thanking them all for their hearty cooperation.

Under the direction of Dr. Blanck the board introduced two new sections in *The Journal*, namely "Book Reviews" and "New Books". Under the former such books as are of particular interest to readers and members will be reviewed in detail, while under the latter new books treating of subjects related to the field covered by *The Journal* will be briefly outlined. The leading scientific book publishers of this country have indicated their willingness to cooperate in this new undertaking and it will be the policy of this board during the ensuing year to secure the cooperation of publishers in foreign countries.

There appears to be doubt among some of the members, as well as the readers of *The Journal*, as to what papers are considered "Contributed Papers". With the idea of clearing up this point, the chairman wishes to present the policy of the board in regard to just what material is acceptable. In the past, that is before 1927, papers as well as the reports of referees and associate referees were published as part of the "Proceedings". Those papers dealing with results of original investigations presented at the annual meeting of the association were, by action of the board, placed in the "Contributed Papers Section" when of special merit. At the meeting of the board last year it was decided to modify this policy so that all papers presented before the meeting, other than reports of referees and associate referees, would be published as contributed papers when dealing with results of original research, subject to the approval of the board. When papers are short or general in nature and only of interest to the meeting for purpose of inducing discussion, they will be published as "Notes" or be released for publication elsewhere.

Furthermore, it is to be noted that the section, "Contributed Papers", offers an outlet for publication of papers other than those presented before the meeting, and it is the desire of the board to induce the publication of results of original investigation in the whole field of agricultural chemistry. Preference, of course, will be given to those papers dealing with analytical methods, but this in no wise excludes the acceptance of a wide range of subject matter. The chairman wishes to impress upon the members of the association the opportunity offered by *The*

Journal for publication of their results and to invite all chemists to make use of these facilities.

With regard to the subscriptions to *The Journal*, the average circulation for the past six years has been as follows: For 1923, 846; for 1924, 838; for 1925, 856; for 1926, 856; for 1927, 847; and for 1928, 847. The complete figures for this period are as set forth below.

DATE	DOMESTIC	ADS	FOREIGN	EXCHANGE	COMPLI- MENTARY	CANADA	ISLANDS	TOTAL
Feb. 1923	655	4	119	..	15	34	15	842
May " "	669	4	121	..	16	36	15	861
July " "	669	4	123	..	16	32	15	859
Oct. " "	642	4	116	..	16	31	12	821
May 1924	647	4	131	..	16	33	12	843
Oct. " "	631	4	135	8	11	31	13	833
May 1925	637	4	147	9	12	32	13	854
Oct. " "	634	4	151	10	13	32	13	857
Jan. 1926	635	4	151	14	13	30	13	860
Aug. " "	620	4	156	14	13	31	13	851
Dec. " "	627	4	156	14	12	31	14	858
May 1927	608	4	166	15	12	32	13	850
Sept. " "	603	4	165	16	12	33	12	845
March 1928	599	4	175	16	12	34	13	853
June " "	601	4	168	16	12	33	12	846

From these figures it is seen that the average number of copies mailed from this office for the period is 848, the highest being 861, in 1923, for a single issue. This year's list averages 850 for the four issues. This number is considered very good when it is known that the records are approximately current; that is, practically all bills now outstanding are for Volume 11, with the exception of a few for single numbers. In 1927, 23 subscriptions for Volume 9 and 8 subscriptions for Volume 10, which were not paid, were taken from the mailing list. In 1928 one subscription to Volume 9, 20 subscriptions to Volume 10, and 2 for Volume 11 were taken from the mailing list and listed as "bad bills". This is in line with our policy of dropping unpaid subscriptions at the end of one year and will explain the slight decrease.

The Board of Editors regrets to announce the loss of the services of one of its staff, Mrs. McCoy. Attention to the many details of the office has always been her first consideration, and she has always been more than efficient. She will soon make her home in Atlanta, Georgia, where her husband's duties take him. Best wishes are extended for success and happiness in her new surroundings.

Plans are under consideration whereby the number of subscriptions may be materially increased during the ensuing year and the scope and usefulness of *The Journal* enlarged.

R. B. DEEMER.

F. C. BLANCK.

H. D. HASKINS.

H. R. KRAYBILL.

W. F. HAND.

REPORT OF COMMITTEE ON QUARTZ PLATE STANDARDIZATION AND NORMAL WEIGHT.

At the last meeting of the association a paper, entitled "Preliminary Report on the Normal Weight of Sucrose for Ventzke-Scale Saccharimeters", was presented by Browne and Zerban¹ in which the previously established value of Bates and Jackson of 99.895° for the polarization of the normal weight of 26 grams of chemically pure sucrose was fully confirmed. Since the making of this preliminary report the papers of this collaborative research by Balch and Hill of the Carbohydrate Laboratory of the Bureau of Chemistry and Soils and by Zerban, Gamble, and Hardin of the New York Sugar Trade Laboratory, Inc., have been completed. The results were presented at the recent meeting of the Sugar Division of the American Chemical Society at Swampscott.

As these papers are published in this number of *The Journal* of the association (see p. 106) it will not be necessary to make further reference to them at this time, except to state that the average results of the determinations obtained at the Bureau of Standards, the Bureau of Chemistry and Soils, and the New York Sugar Trade Laboratory for the polarization of 26 grams of chemically pure sucrose under the prescribed conditions is 99.904° , a value which can be rounded out to the even decimal 99.9 without sensible error. In accordance with this calculation it would require $\frac{26 \times 100}{99.9} = 26.026$ grams of chemically pure sucrose to give a reading of 100° upon saccharimeters provided with the German sugar scale, whose 100° point is standardized according to the Herzfeld-Schönrock factor of 34.657 angular degrees for the *D* line of sodium light. This corrected calculated value for the normal weight has been confirmed experimentally by Balch and Hill. (See p. 110.)

RECOMMENDATIONS.

The committee offers the following recommendations:

(1) That for purposes of research and of commercial analysis, in the case of refined sugars and other products of high purity, a normal weight of 26.026 grams be employed in place of the present weight of 26 grams for all saccharimeters whose 100° point has been established according to the Herzfeld-Schönrock factor.

(2) That for purposes of research, in the case of sugar products of low purity where other errors exist, due to the volume of the lead precipitate or to other causes, a correction for all such errors be introduced at the same time as the proposed correction in the value of the normal weight.

(3) That in this connection the question of the extent of the errors due to the clarification of impure sugar solutions with lead subacetate

¹ *This Journal*, 1928, 11: 55.

and other agents and to all other causes in the polarimetric analysis of sugar products be referred to the present Committee on Quartz Plate Standardization and Normal Weight for collaborative investigation.

FREDERICK BATES.
C. A. BROWNE.
F. W. ZERBAN.

Approved.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS.

The committee recommends that the definitions and interpretation of terms be printed with the official methods and changes in methods before they are published in the revised editions of *Methods of Analysis*. It will be noted that definitions 3 and 4 were not approved for final acceptance.

The following definitions and interpretation of terms are recommended :

For Final Adoption as Official.

1. MAXIMUM AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE.

The *chlorine* in mixed fertilizers in which the potash is claimed as sulfate shall not exceed five-tenths of one per cent (0.5%) more than what is called for in the minimum potash content based on the definition for sulfate of potash as formulated by the committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.5.

2. DEFINITION OF PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALI SALTS CONTAINING POTASH.

These products are *not* potassium phosphate. They may be called non-acid phosphates with potash.

3. MURIATE OF POTASH (COMMERCIAL POTASSIUM CHLORIDE).

Muriate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as chloride. (Not approved.)

4. SULFATE OF POTASH (COMMERCIAL POTASSIUM SULFATE).

Sulfate of potash is a potash salt containing not less than forty-eight per cent (48%) of potash (K_2O) largely as sulfate, and not more than two and one-half per cent (2.5%) of chlorine. (Not approved.)

5. UNLEACHED WOOD ASHES.

Unleached wood ashes are ashes that result from burning unleached wood, that have had no part of their plant food removed, and that contain four per cent (4%) or more of water-soluble potash (K_2O).

6. NITRATE OF SODA (COMMERCIAL SODIUM NITRATE).

Nitrate of soda is commercial sodium nitrate containing not less than fifteen per cent (15%) of nitrogen, chiefly as sodium nitrate.

7. KAINIT.

Kainit is a potash salt containing potassium and sodium chlorides and sometimes sulfate of magnesia with not less than twelve per cent (12%) of potash (K_2O).

8. DRIED BLOOD.

Dried blood is the collected blood of slaughtered animals, dried and ground and containing not less than twelve per cent (12%) of nitrogen in organic forms.

9. FERTILIZER GRADE.

The *grade of a fertilizer* shall represent the minimum guarantee of its plant food expressed in terms of nitrogen, available phosphoric acid, and water-soluble potash.

Second Recommendation as Tentative.

1. GROUND STEAMED BONE.

Ground steamed bone is a product resulting from grinding animal bones that have been previously steamed under pressure.

2. GROUND RAW BONE.

Ground raw bone is a product resulting from drying and grinding animal bones that have not been previously steamed under pressure.

3. TANKAGE.

This term (without qualification) shall be restricted to meat and bone tankage derived from the rendered, dried, and ground by-products from the slaughter of animals, or from carcasses of animals that have died otherwise than by slaughter.

4. FISH TANKAGE, FISH SCRAP, DRY GROUND FISH.

Fish tankage, fish scrap, dry ground fish is the dried ground product derived from rendered or unrendered fish.

5. GARBAGE TANKAGE.

Garbage tankage is the rendered, dried, and ground product derived from waste household food materials.

6. CRUDE, INERT, OR SLOW-ACTING NITROGENOUS MATERIALS.

Crude, inert, or slow-acting nitrogenous materials are unprocessed organic substances relatively high in nitrogen but having a very low value as plant food and showing a low activity by both the alkaline and neutral permanganate methods [below fifty per cent (50%) and eighty per cent (80%), respectively].

7. PROCESS TANKAGES.

Process tankages are the products made from crude inert nitrogenous materials by processing under steam pressure, with or without the use of acids, for the purpose of increasing the activity of the nitrogen.

These products shall not be called "tankages" without proper qualification.

8. HOOF AND HORN MEAL.

Hoof and horn meal is a product resulting from the processing, drying, and grinding of hoofs and horns.

9. SUPERPHOSPHATE.

Superphosphate is the ground product resulting from mixing finely ground rock phosphate and sulfuric acid or phosphoric acid. The grade should always be used as a prefix to the name. Example: 16% Superphosphate.

It is recommended that the use of the term "Acid Phosphate" be discontinued.

First Recommendation as Tentative.

1. ORDER OF TERMS.

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third.

2. STATEMENT OF GUARANTEES.

It is recommended and urged that the *statement of guarantees* of mixed fertilizers be given in whole numbers and without fractions.

3. ACIDULATED FISH TANKAGE, FISH SCRAP, DRY GROUND FISH.

Acidulated fish tankage, fish scrap, dry ground fish is the rendered, dried, and ground product derived from fish and with or without treatment with sulfuric acid.

4. SIGNIFICANCE OF THE NAME OF A MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER.

When the name of a material is used as a part of the brand name of a mixed fertilizer, as for example blood, bone or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a material is used as a brand or as part of a brand the word "brand" shall follow the name of the material. Example: "Fish Brand Fertilizer".

5. AMMONIATED SUPERPHOSPHATE.

Ammoniated superphosphate is that class of product containing both dissolved phosphate (superphosphate or dissolved bone) and nitrogenous compounds, but without the addition of potash.

6. ACTIVATED SEWERAGE PRODUCTS.

Activated sewerage products are made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried in rotary kilns, ground, and screened.

Proposed for Future Consideration.

1. Definition of product from wool carding establishments largely sheep manure. Seeds from plants and wool fiber.

2. *Agricultural Lime*.—As this term is indiscriminately applied not only to a great variety of products originating in the lime quarry but also to marl, shell lime, and refuse lime products from various industries, it is recommended that the use of the term be discontinued and that each specific lime product used as a soil amendment be defined.

3. *Quicklime, Burned Lime, Caustic Lime, Lump Lime, Unslaked Lime*.—This is commercial calcium oxide or a mixture of calcium oxide with varying smaller quantities of magnesium oxide resulting from heating suitable calcium-containing minerals until substantially all the carbon dioxide has been eliminated. With a pure limestone the oxides should total 99 per cent.

4. *Hydrated or Slaked Lime*.—This is the product obtained by treating quick lime with sufficient water or steam to combine with its oxides. It usually contains about 65 per cent of calcium oxide or an equivalent of magnesium oxide.

5. *Air-Slaked Lime*.—This is the product obtained by exposing caustic lime to the atmosphere, whereby it absorbs both moisture and carbon dioxide. It usually consists of a mixture of calcium oxide, calcium hydroxide, and calcium carbonate, or of these with smaller and varying quantities of the corresponding magnesium compounds.

6. *Ground Limestone*.—This is the product obtained by grinding calcitic or dolomitic limestone. Seventy-five per cent or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than 45 per cent of calcium oxide or the mixed oxides of calcium and magnesium.

7. *Ground Shell Lime*.—This is the product obtained by grinding the shells of mollusks. Seventy-five per cent or more should pass a 100-mesh sieve and should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.

8. *Marl, Ground Shell Marl*.—This is the product obtained by grinding natural deposits of shell marl. Seventy-five per cent or more should pass a 100-mesh sieve. It should contain calcium and magnesium carbonates equivalent to not less than 40 per cent of calcium oxide or the mixed oxides of calcium and magnesium.

9. *Waste Lime, By-Product Lime*.—This is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by the prefixation of the name of the industry or process by which it is produced, i. e., gas-house lime, tanners' lime, acetylene lime waste, lime-kiln ashes, etc.

10. *Calcium Sulfate, Gypsum*.—This is a neutral salt of calcium sulfate. It is accompanied by varying quantities of impurities including about 20 per cent of water. It does not neutralize acid solutions.

11. *Available Phosphoric Acid*.—This is the sum of the water-soluble and the citrate-soluble phosphoric acid, ordinarily termed available phosphoric acid.

12. *High-Analysis Fertilizer*.—A high-analysis fertilizer is a commercial fertilizer containing 30% or more of phosphoric acid, potash or nitrogen, alone or in combination.

13. *Soil Amendment*.—A soil amendment is any substance which is added to the soil for the purpose of improving its physical or chemical character or promoting the growth of crops, exclusive of commercial fertilizer and barnyard manure.

14. *Peat*.—This is partly decayed vegetable matter of natural occurrence, composed chiefly of organic matter with some nitrogen of low activity.

15. *Charred Peat*.—This is peat dried at such a temperature as to cause partial decomposition.

16. *Sulfate of Ammonia*.—This is a commercial product composed chiefly of ammonium sulfate and containing 20 per cent or more of nitrogen.

17. Classification of cyanamide and urea as water-soluble non-proteid organic material.

C. H. JONES.

H. D. HASKINS.

R. N. BRACKETT.

J. W. KELLOGG.

G. S. FRAPS.

Approved.

REPORT OF COMMITTEE ON REVISION OF METHODS OF SOIL ANALYSIS.

The four recommendations of the General Referee on Soils and Liming Materials are approved.

The committee recommends further that the following method for the determination of soil reactions be published in *Methods of Analysis* as Paragraph 33 at the end of the chapter "Soils—Tentative":

SOIL REACTION¹.

METHOD OF STATEMENT.

In the interest of simplicity and ease of interpretation, use a dual system of statement, giving both pH values and their equivalents in arithmetically related numbers, as tabulated below:

SÖRENSEN OR pH VALUES	SPECIFIC ACIDITY OR HYDROGEN-ION CONCENTRATIONS	SÖRENSEN OR pH VALUES	SPECIFIC ALKALINITY OR HYDROXYL-ION CONCENTRATIONS
7.0	0.0	7.0	0.0
6.9	0.5	7.1	0.5
6.8	1.0	7.2	1.0
6.7	1.5	7.3	1.5
6.6	2	7.4	2
6.5	3	7.5	3
6.4	4	7.6	4
6.3	5	7.7	5
6.2	6	7.8	6
6.1	8	7.9	8
6.0	10	8.0	10
5.9	12.5	8.1	12.5
5.8	16	8.2	16
5.7	20	8.3	20
5.6	25	8.4	25
5.5	31.5	8.5	31.5
5.4	40	8.6	40
5.3	50	8.7	50
5.2	63	8.8	63
5.1	80	8.9	80
5.0	100	9.0	100
4.9	125	9.1	125
4.8	160	9.2	160
4.7	200	9.3	200
4.6	250	9.4	250
4.5	315	9.5	315

DETERMINATION.

Use either the colorimetric or electrometric method, as convenient. Determine the reaction values on fresh moist samples, using a soil to water ratio of 1 : 5, with intermittent agitation for 30 minutes.

W. H. MACINTIRE.

A. G. MCCALL.

A. W. BLAIR.

J. S. MCHARGUE.

J. A. BIZZELL.

Approved.

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES.

The report of this committee is, as usual, very adequately presented in the separate reports of Subcommittees A, B and C. In addition, however, it is desired to call the attention of the association to the generous cooperation of referees and associate referees and to the general excellence of their contributions. Progress in our unique and important

¹ E. T. Wherry. *J. Wash. Acad. Sci.*, 1919, 9: 305; 1921, 11: 197; *Ecology*, 1922, 3: 346; *Am. J. Pharm.*, 1927, 2: 59.

field of endeavor depends almost entirely upon the sustained and devoted efforts of these men and women.

Nor should the improvement in the presentation of papers on the floor of the convention be allowed to pass unnoticed. The pains taken to illustrate features by means of slides where possible, the omission of details which cannot be readily followed by the audience, and the attention given to clarity of expression, have all contributed much to the spirit and interest of these proceedings.

It is necessary again to urge upon referees and associates that their reports be submitted in duplicate and in time for consideration. Although in the main these points are observed, yet the work of the several subcommittees is, at times, greatly hampered by failure to comply with this request, which is each year clearly set forth in the call of the secretary for the annual meeting.

About two years ago this committee called attention to the growing importance of microchemical methods and suggested that referees and associate referees consider such methods wherever it appeared that they might be used to advantage. The admirable address of the president this year again emphasizes this point and suggests the opportunity for enlargement upon this phase of study of methods. The most desirable plan for approaching this undertaking is a question to which the committee has given some thought but upon which it is not prepared at this time to make specific recommendations. Meanwhile suggestions from referees and other members interested will be welcomed.

E. M. BAILEY.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By J. W. KELLOGG (Department of Agriculture, Harrisburg, Pa.),
Chairman.

WATERS, BRINE, AND SALT.

No report was submitted.

TANNING MATERIALS AND LEATHERS.

No report was submitted.

INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the tentative method for the determination of copper in Bordeaux Paris green and Bordeaux calcium arsenate¹ be recommended

¹ *Methods of Analysis*, A. O. A. C., 1925, 63, par. 87.

as official¹ after the following revision has been made (first action): For the second sentence substitute the following sentence: "Add 25 cc. of hydrogen peroxide solution; dilute to about 100 cc.; and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2-3 amperes".

Approved.

(2) That the tentative method for the determination of unsulfonated residue in mineral oils and in recovered oils obtained in the analysis of oil emulsions, as amended at the 1927 meeting², be adopted as official (final action).

Approved.

FLUORINE COMPOUNDS.

It is recommended that the studies of methods of analysis for fluorine compounds be continued.

Approved.

CAUSTIC POISONS.

It is recommended—

(1) That the method for the estimation of benzophenol described by Chapin³ be subjected to further collaborative study.

Approved.

(2) That the effect of salicylates on the Chapin method be studied, and that the procedure for their removal be tested by collaborative work.

Approved.

SOILS AND LIMING MATERIALS.

REACTION VALUE OF SOILS.

Referred to Committee on Revision of Soil Analysis, W. H. MacIntire, chairman, according to a recommendation adopted in 1926⁴.

LIMING MATERIALS.

It is recommended—

(1) That the sugar method for the determination of the caustic value of lime⁵ be adopted as official (final action).

Approved.

(2) That the referee report next year on the subject of the solubility of soil potassium⁶ and suggest either continuance of the work or such other disposition of the subject as may be deemed advisable.

Approved.

¹ *This Journal*, 1924, 7: 261.

² *Ibid.*, 1928, 11: 65.

³ U. S. Dept. Agr. Bull. 1308.

⁴ *This Journal*, 1927, 10: 62.

⁵ *Ibid.*, 1928, 11: 153.

⁶ *Ibid.*, 65.

LESS COMMON METALS IN SOILS.

(Referred to Committee on Revision of Soil Analysis.)

It is recommended—

(1) That the study of methods for the determination of less common metals in soils be continued and that the determination of boron be considered.

Approved.

(2) That the Associate Referee on the Determination of Fluorine (under Insecticides and Fungicides) be requested to adapt his findings so as to provide a method for the determination of fluorine in soils.

Approved.

FEEDING STUFFS.

STOCK FEED ADULTERATION.

It is recommended—

(1) That no change be made in the tentative method adopted last year for the detection of dried buttermilk in feeding stuffs.

Approved.

(2) That the referee send out samples of hoof-meal mixtures for preliminary collaborative work and obtain suggestions and criticisms of the hoof-meal method as presented by the Referee on Feeding Stuff.

Approved.

MINERAL MIXED FEEDS.

It is recommended—

(1) That the proposed method for the determination of lime in mineral feeds be further studied and that the acetic acid modification of this method be tried for comparison of results.

Approved.

(2) That the proposed method for the determination of iodine in mineral feeds, published in the 1926 report¹, be further studied and that consideration also be given to other proposed methods.

Approved.

(3) That methods for the determination of iodine in organic minerals be studied.

Approved.

DETERMINATION OF MOISTURE.

It is recommended—

(1) That the toluene distillation method (Bidwell-Sterling²) for the determination of moisture be adopted as official (final action). First action was taken in 1926.

Approved.

¹ *This Journal*, 1927, 10: 176.

² *Ibid.*, 1926, 9: 30.

(2) That studies of methods for the determination of moisture in such materials as molasses feed and linseed meal be continued.

Approved.

SUGARS AND SUGAR PRODUCTS.

HONEY.

It is recommended that attempts to establish an acceptable levulose-dextrose ratio, which can be applied to honey as an index of purity without injustice to the legal yet seemingly abnormal honey, be temporarily abandoned for studies which shall have for their objects critical reviews of existing methods for determining dextrose on the one hand and levulose on the other when they occur in the presence of one another. A method claimed to be selective towards levulose is that of Nyn, studies on which were recommended last year. This study is in progress in the laboratory of the associate referee and, it is understood, elsewhere.

Approved.

MAPLE PRODUCTS.

No report was submitted.

STARCH CONVERSION PRODUCTS.

No associate referee was appointed.

It is recommended that this subject be continued.

DRYING, DENSIMETRIC AND REFRACTOMETRIC METHODS.

It is recommended—

(1) That studies of these methods be continued.

Approved.

(2) That the refractometric method receive particular attention—

(a) by a survey of the tables of refractive indices corresponding to sugar concentration, and

(b) a study of the influence of known amounts of impurities upon the refractive index of sugar solutions.

Approved.

POLARISCOPIC METHODS.

It is recommended—

(1) That this year's work be repeated, and that sucrose alone, pure invert sugar alone and mixtures of the two, with and without the further addition of aspartic acid or asparagine, be used.

Approved.

(2) That the isolation in the pure state of the amids and the amino acids occurring in cane blackstrap molasses be attempted.

Approved.

CHEMICAL METHODS FOR REDUCING SUGARS.

It is recommended—

(1) That the official volumetric thiosulfate method¹, p. 191, par. 37, be revised as recommended by the associate referee (first action). (See p. 38.)

Approved.

(2) That the last two lines of par. 38, p. 192, be revised as follows: "under 37, beginning with 'Cool, and add strong sodium hydroxide solution' " (first action).

Approved.

(3) That the first paragraph under "Determination", Lane-Eynon general volumetric method², be amended as recommended by the associate referee. (See p. 38.)

Approved.

(4) That the referee give attention next year to Recommendations 2, 3, 4 and 5³ and either make suggestions for further study or dispose of them as deemed advisable.

Approved.

FERTILIZERS.

PHOSPHORIC ACID.

It is recommended—

(1) That the words "Nearly neutralize with strong hydrochloric acid", line 12, in the gravimetric determination of phosphoric acid (I, 7)⁴ be changed to read, "Neutralize with strong hydrochloric acid, using litmus paper or bromthymol blue as indicator" (final action).

Approved.

(2) That the direction to "burn first at a low heat and then ignite intensely until white or grayish white", line 17, in the gravimetric determination of phosphoric acid (I, 7)⁴ be changed to read "burn first at a low heat and ignite to constant weight preferably in an electric furnace, at 950°–1000°C." (final action).

Approved.

(3) That a third alternative method for the preparation of magnesia mixture [I, 5 (c)]⁵ be worded as follows (final action):

(3) Dissolve 55 grams of crystallized magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using at the rate of 15 cc. per 100 cc. of solution.

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 191.

² *This Journal*, 1926, 9: 35.

³ *Ibid.*, 1928, 11: 67.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 3.

⁵ *Ibid.*, 2.

(4) That the work be continued next year to include a study of the volumetric analysis of phosphates in the presence of sulfates and organic matter.

Approved.

(5) That further study be made of the effect of sulfates on the volumetric method for phosphoric acid, and that smaller amounts of sulfates be used with a view to deleting the precipitation at 45°–50°C.

Approved.

NITROGEN.

It is recommended—

(1) That further collaborative work be done comparing the Robertson method, as given by the associate referee, with the Jones¹ method for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea.

Approved.

(2) That the official zinc iron method for the determination of nitric and ammoniacal nitrogen² be discarded (final action).

Approved.

(3) That the official reduced iron method for the determination of nitric and ammoniacal nitrogen³ be marked "Applicable only in the absence of cyanamide and urea" (final action).

Approved.

NITROGEN ACTIVITY METHODS.

It is recommended—

(1) That in Par. 38, line 3³, the word "water-insoluble" be inserted before the word "nitrogen", so as to have the phrase read, "making a correction for the water-insoluble nitrogen of the filter, if necessary" (final action).

Approved.

(2) That in Par. 40³, the directions for the preparation of alkaline permanganate solution be changed to read as follows: "Dissolve 25 grams of potassium permanganate in hot water and, separately, 150 grams of sodium hydroxide in cold water; combine the solutions when cold; and dilute to 1 liter. Discard any permanganate solutions that have become green in color" (final action).

Approved.

(3) That in Par. 41 (a)⁴, after line 3, the following statement be added: "When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed" (final action).

Approved.

¹ *This Journal*, 1928, 11: 32.

² *Methods of Analysis*, A. O. A. C., 1925, 11.

³ *Ibid.*, 12.

⁴ *Ibid.*, 13.

(4) That in Par. 42, line 3¹, after the sentence, "transfer from the filter to a 500-600 cc. Kjeldahl distillation flask", the following phrase be added: "loosening adhering particles by rubbing gently with a stiff brush but avoiding the transfer of portions of the brush or of paper fibers" (final action).

Approved.

(5) That in Par. 42, line 4, in place of "a piece of paraffin the size of a pea", the following phrase be substituted, "a drop of mineral lubricating oil weighing not more than 50 mg." (final action).

Approved.

(6) That in Par. 42, line 5, the sentence, "Connect with, etc.", be changed to read as follows: "Connect with an upright condenser to the lower end of which has been attached a 100 cc. graduated cylinder containing standard acid, and so arranged as to receive the distillate below the surface of the acid or otherwise so trapped as to prevent loss of ammonia fumes" (final action).

Approved.

(7) That in Par. 42, line 7, the words "at least" be deleted from the sentence, "Digest slowly with a very low flame, for at least 30 minutes, below distillation point", and that the phrase "below distillation point" be changed to read "barely below distillation point" (final action).

Approved.

(8) That in Par. 42, line 9, the direction to "distil until 95 cc. of distillate is obtained", be changed to read as follows: "Distil 95 cc. in 60 minutes (plus or minus 5 minutes), controlling the distillation so that approximately 24 cc. of distillate is obtained in each 15 minute period. Conduct the first part of the distillation over a bare flame but use wire gauze 10 minutes before completion to avoid breaking the flask". Also that before the direction to "Titrate with standard alkali" there be inserted the added direction to "transfer the distillate to an Erlenmeyer flask or to a beaker" (final action).

Approved.

(9) That to Par. 42, the following directions be added: "If the active water-insoluble nitrogen is found to be less than 55 per cent of the total water-insoluble organic nitrogen present, it is recommended that a second portion of the sample be prepared as directed under 41 (a). Dry the residue below 80°C., transfer from the filter to a Kjeldahl flask as directed above, and determine the nitrogen as directed under 19 or 22. Recalculate the percentage of active water-insoluble nitrogen on the basis of the quantity of water-insoluble nitrogen thus found" (final action).

Approved.

(10) The committee recognizes and commends the excellent work done

¹ *Methods of Analysis*, A. O. A. C., 1925, 13.

by the Associate Referee on Nitrogen Activity Methods in Fertilizers but feels that intensive study of this subject is of doubtful wisdom and questionable value and, therefore, recommends that the work be continued with the view to a final report next year.

Approved.

POTASH.

It is recommended—

(1) That the study of the use of calcium carbonate in preparing the solution for the determination of potash be continued.

Approved.

(2) That the method submitted by the associate referee and adopted as tentative last year for the direct titration of chlorine in mixed fertilizers¹ be adopted as official (first action).

Approved.

(3) That the associate referee continue the study of methods for the determination of potash in mixed fertilizers.

Approved.

PLANTS.

PREPARATION OF PLANT MATERIALS FOR ANALYSIS.

It is recommended that this work be continued.

LESS COMMON METALS IN PLANTS.

It is recommended—

(1) That the methods for the determination of the following rare elements in plants: manganese, copper and zinc, as submitted by the associate referee, be adopted as tentative (see p. 35).

Approved.

(2) That these methods be further studied next year for the purpose of adopting them as official if the results warrant such action.

Approved.

TOTAL CHLORINE IN PLANTS.

It is recommended—

(1) That further collaborative work be undertaken on methods for the determination of chlorine in plant material.

Approved.

(2) That further work be done investigating the possibilities of the "open Carius method" for the determination of chlorine in plant material.

Approved.

(3) That future work include investigations to ascertain whether or not organic chlorine can be determined by ashing methods.

Approved.

¹ *This Journal*, 1928, 11: 34.

(4) That consideration be given to the study of methods for the determination of carbohydrates and for the various forms of nitrogen in plants.

Approved.

IRON AND ALUMINA IN PLANTS.

It is recommended that collaborative work be undertaken on a micro-chemical method for the determination of iron and alumina in plants.

Approved.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By A. G. MURRAY (Food, Drug and Insecticide Administration, Department of Agriculture, Washington, D. C.), *Chairman*.

SPECIFIC GRAVITY AND ALCOHOL.

In accordance with last year's recommendations the associate referee obtained the opinion of a considerable number of members of the association on the desirability of changing the alcohol tables at present official from the true to the apparent basis and from $20^{\circ}/4^{\circ}$ to $15.56^{\circ}/15.56^{\circ}$, $20^{\circ}/20^{\circ}$ or $25^{\circ}/25^{\circ}$. The committee considers it inadvisable to adopt any change until such time as it is practicable to make available suitable tables.

It is recommended, therefore, that the Bureau of Standards be requested to calculate tables for the apparent specific gravity of alcohol at $15.56^{\circ}/15.56^{\circ}$, $20^{\circ}/20^{\circ}$, and $25^{\circ}/25^{\circ}$ with a view to incorporating them in *Methods of Analysis* when this book is republished, at which time it is proposed to recommend for formal adoption by the association these revised tables as a substitute for the tables at present official.

Approved.

SPICES AND OTHER CONDIMENTS.

No report was submitted. It is recommended that the recommendations¹ of last year be continued.

Approved.

NAVAL STORES.

TURPENTINE.

It is recommended that the tentative sulfuric-fuming nitric acid method for determining mineral oil in turpentine², as modified by the associate referee, be further studied, with particular reference to the

¹ *This Journal*, 1925, 8: 264.

² *Methods of Analysis*, A. O. A. C., 1925, 409.

means of distinguishing between the normal residue from pure turpentine and that from a slightly adulterated turpentine.

Approved.

DRUGS.

ALCOHOL IN DRUGS.

It is recommended—

(1) That the method described by the associate referee for the determination of methyl and ethyl alcohols in the presence of each other when methyl alcohol is present in small quantities (5 per cent or less) be made tentative.

Approved.

(2) That further study of this subject be transferred to the Referee on Beers, Wines and Distilled Liquors.

Approved.

ARSENICALS.

It is recommended that the method proposed by the associate referee for the determination of arsenic in sodium cacodylate¹ in 1926 be made official (final action).

Approved.

CHLOROFORM AND CARBON TETRACHLORIDE.

It is recommended—

(1) That the method for the determination of chloroform and carbon tetrachloride described by the associate referee be adopted as tentative, superseding the present tentative method².

Approved.

(2) That no further work on this subject be done.

Approved.

RADIOACTIVITY IN DRUGS AND WATER.

It is recommended that work on radioactivity in drugs and water be continued as outlined in Recommendation 2 of 1924³.

Approved.

LAXATIVES AND BITTER TONICS.

It is recommended that the methods outlined by the associate referee be further studied in conjunction with pharmacological experiments.

Approved.

MERCURIALS.

It is recommended—

(1) That the present tentative method for the determination of

¹ *This Journal*, 1928, 11: 48.

² *Ibid.*, 1927, 10: 45.

³ *Ibid.*, 1925, 8: 267.

calomel in tablets¹ be amended to permit the use of asbestos as a substitute for paper in filtering.

Approved.

(2) That the method proposed by the associate referee for the determination of mercurous iodide be adopted as tentative.

Approved.

(3) That work on mercurials be continued as recommended by the referee.

Approved.

MICROCHEMICAL METHODS FOR ALKALOIDS.

It is recommended—

(1) That the microchemical tests and descriptions for the identification of quinine, quinidine, cinchonine, and cinchonidine described by the associate referee be adopted as tentative.

Approved.

(2) That the microchemical tests for the identification of atropine and pilocarpine submitted in 1927² be adopted as tentative.

Approved.

(3) That other important alkaloids be further studied with a view to including them in a scheme for identification by microchemical methods.

Approved.

TERPIN HYDRATE.

It is recommended that study of analytical methods for the determination of terpin hydrate be continued.

Approved.

SANTONIN.

It is recommended that the study of analytical methods for the determination of santonin be continued, that consideration be given to the methods mentioned previously by Palkin³, and that if necessary an attempt be made to devise a new method or new methods.

Approved.

ETHER.

It is recommended that the method described by the associate referee for the determination of ether be studied collaboratively with a view to its adoption.

Approved.

BIOASSAY OF DRUGS.

It is recommended that the paper presented this year on the bioassay of drugs be referred to a referee for study next year.

Approved.

¹ *This Journal*, 1928, 11: 51.

² *Ibid.*, 354.

³ *Ibid.*, 1926, 9: 326.

GINGER FLUIDEXTRACT.

It is recommended that analytical methods for the examination of ginger fluidextract be studied.

Approved.

EPHEDRA.

It is recommended that further study of the method described by the associate referee for the determination of ephedra be undertaken with a view to its adoption.

Approved.

PILOCARPINE.

It is recommended that the method described by the associate referee for the determination of pilocarpine in which methyl red is used as an indicator be adopted as tentative.

Approved.

THYMOL.

It is recommended—

(1) That the method described by the associate referee for the determination of thymol be adopted as tentative.

Approved.

(2) That further work on the quantitative determination of thymol in mixtures be undertaken.

Approved.

MENTHOL.

It is recommended that the method proposed by the associate referee for the determination of menthol be further studied with a view to its adoption.

Approved.

CHLORIDES AND BROMIDES.

It is recommended—

(1) That the method described by the associate referee for the determination of bromide in the presence of chloride be subjected to collaborative study.

Approved.

(2) That the problem of the separation of the three halogens, chlorine, bromine, and iodine by chemical means be further studied.

Approved.

(3) That the applicability of potentiometric methods for the determination of chlorides and bromides be studied.

Approved.

CHENOPODIUM OIL.

It is recommended that the method described by the associate referee for the determination of chenopodium oil be further studied collaboratively with a view to adoption.

Approved.

SABADILLA.

It is recommended that no action be taken on the method proposed for the determination of sabadilla in view of the fact that it is essentially a U. S. P. type of procedure.

Approved.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By H. A. LEPPER (Food, Drug and Insecticide Administration, Washington, D. C.), *Acting Chairman*.

DAIRY PRODUCTS.

BUTTER.

No report was submitted.

It is recommended—

- (1) That work be continued as outlined last year¹.

Approved.

- (2) That the mixing modification be removed from the present official method for preparing a sample of butter², as recommended by the associate referee, by deletion of the words "or mix", line 2 (final action).

Approved.

CHEESE.

It is recommended—

- (1) That the phrase "* * * then add 2.5 cc. of the permanganate solution * * *", 19th line in the tentative method for the quantitative determination of citric acid³, be corrected to read "* * * add 25 cc. of the permanganate solution * * *", since the volume of permanganate solution stated in the report is obviously insufficient.

Approved.

- (2) That the Schmidt-Bondzynski method for the determination of fat in cheese⁴ be changed by substituting for the phrase "* * * 0.5 gram of sand to prevent bumping * * *" the phrase "* * * about 0.5 gram of sand, previously digested with concentrated hydrochloric acid * * *" (first action).

This recommendation does not modify the method, but it provides a means of avoiding difficulty with ferric chloride contamination of the fat.

Approved.

- (3) That the tentative methods for the determination of tartaric and citric acids in cheese given in the report of the associate referee for 1927⁵,

¹ *This Journal*, 1928, 11: 75.

² *Methods of Analysis*, A. O. A. C., 1925, 276.

³ *This Journal*, 1928, 11: 42.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 279.

⁵ *This Journal*, 1928, 11: 40.

with the correction noted in Recommendation (2), be studied collaboratively.

Approved.

(4) That further work be done on the phosphorus pentoxide: calcium oxide ratios of processed cheese.

Approved.

(5) That the recommendation of 1926 for the study of methods for the detection of preservatives, coloring matters, emulsifying agents, or other added substances in cheese be repeated.

Approved.

MALTED MILK.

It is recommended—

(1) That the study of methods for the determination of lactose in malted milk be continued and that other sugars be included in the study.

Approved.

(2) That the associate referee submit the method for the microscopic identification of malted milk mentioned in his report to collaborative study.

Approved.

DRIED MILK.

No report was submitted.

It is recommended—

(1) That methods for the determination of various sugars in mixtures containing dried milk and malted milk be referred to the Associate Referee on Malted Milk for study.

Approved.

(2) That the associate referee study methods for the determination of total fat in dried milk.

Approved.

ICE CREAM.

No report was submitted.

It is recommended—

(1) That the following recommendation, adopted last year, be repeated: "That owing to the uncertainty of the nature of the work contemplated in the following recommendations of the associate referee (1927), he discuss plans for future work with the General Referee on Dairy Products: That the proposed method for ash in ice cream be submitted to further collaborative study in connection with its value as a basis for the calculation of milk solids not fat, and further, that a collaborative study be made of the average composition of normal milk in an effort to produce all available data on the subject, as well as to establish ratios as a basis for the calculation of milk solids not fat in ice cream".

Approved.

(2) That the modified Ferris method for the determination of gelatin in ice cream be submitted to collaborative study.

Approved.

(3) That methods for the determination of milk protein in ice cream be further studied.

Approved.

MILK PROTEINS.

No report was submitted. It is recommended that further study be given to methods for the determination of milk proteins.

Approved.

QUALITATIVE TESTS.

It is recommended that the method for the detection of gelatin be studied collaboratively.

Approved.

FATS AND OILS.

It is recommended—

(1) That the tentative method for the determination of liquid and solid fatty acids¹ be dropped.

Approved.

(2) That the lead-salt-ether method for the determination of saturated and unsaturated fatty acids² be adopted as official (first action). (See p. 44.)

Approved.

(3) That the "cold test" given in the referee's report be adopted as a tentative method and further studied with its adoption as official in view (see p. 46).

Approved.

(4) That study be made of the combined procedure for the determination of the Reichert-Meissl and Polenske values.

Approved.

(5) That a study be made of the official F. A. C. method for unsaponifiable matter with respect to its applicability to cod liver and other fish oils.

Approved.

BAKING POWDER AND BAKING CHEMICALS.

It is recommended—

(1) That the method for the determination of aluminum by precipitation with phenylhydrazine be adopted as a tentative method and further studied (see p. 46).

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 292.

² *This Journal*, 1928, 11: 303.

(2) That the referee consider the need of a continuation of the study of the separation and determination of the different forms of phosphates used as baking acids and report recommending continuation or discontinuance of this subject.

Approved.

EGGS AND EGG PRODUCTS.

TOTAL SOLIDS, FAT, LIPOIDS, AND LIPOID PHOSPHORIC ACID (P_2O_5).

It is recommended—

(1) That methods for the determination of fat (acid hydrolysis), lipoids, and lipid phosphoric acid (P_2O_5) be studied collaboratively.

Approved.

(2) That methods for the determination of total phosphoric acid with consideration of the use of potassium hydroxide as a fixing agent and methods for the determination of added sugars be studied.

Approved.

(3) That in recognition of the referee's report on methods for the determination of moisture in eggs, as recommended in 1926, further collaborative study be made of the 98°C. vacuum oven method, with a view to its adoption as official.

Approved.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH.

It is recommended—

(1) That the method for the determination of ash given in the associate referee's report be adopted as tentative (see p. 55).

Approved.

(2) That the method for the determination of unsaponifiable matter given in the report of the associate referee be adopted as tentative (see p. 56).

Approved.

(3) That the method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol in dried and liquid eggs described as Method 1 in the report of the associate referee be adopted as tentative and studied further (see p. 56).

Approved.

DETECTION OF DECOMPOSITION.

It is recommended—

(1) That the method for the determination of acidity of fat¹ adopted last year as official (first action) be made official (final action).

Approved.

(2) That the method for the determination of acid-soluble phosphoric acid be further studied.

Approved.

¹ *This Journal*, 1927, 10: 50.

(3) That a study be made of methods for determining ammonia nitrogen and reducing substances as dextrose.

Approved.

FOOD PRESERVATIVES.

It is recommended—

(1) That search be continued for a method for detecting saccharin in food products which shall be more expeditious than the present official method and shall not involve the destruction of the saccharin.

Approved.

(2) That the effort be continued to devise a method for the separation and determination of saccharin and sodium benzoate, respectively, when both are present in a food product.

Approved.

(3) That effort be made to formulate satisfactory methods for the detection and separation of hydrogen peroxide and other preservatives as well as sweeteners.

Approved.

COLORING MATTERS IN FOODS.

It is recommended—

(1) That collaborative study be devoted to the quantitative separation of amaranth from tartrazine.

Approved.

(2) That further study be devoted to the elimination or conversion of interfering substances when determining amaranth and tartrazine.

Approved.

(3) That additional work be undertaken to obtain a quantitative estimation of new green FCF and its separation from other permitted dyes.

Approved.

(4) That, as recommended last year, additional work be done on the separation of yellow A B and yellow O B from other oil-soluble dyes.

Approved.

METALS IN FOODS.

It is recommended—

(1) That modifications of the Gutzeit method and of the volumetric and gravimetric methods for arsenic be studied collaboratively.

Approved.

(2) That the thiocyanate colorimetric and the acetate colorimetric methods for lead be studied collaboratively.

Approved.

(3) That the correction of the conditions in the iodine volumetric methods for tin, especially in connection with the Baker-Sellers and the zinc-iron precipitation methods, be studied collaboratively.

Approved.

(4) That the recommendation made last year for further study of methods for the determination of copper and zinc be repeated.

Approved.

FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That the task of making the methods for fruit and fruit products more definite and complete be undertaken when *Methods of Analysis* is revised.

Approved.

(2) That the recommendation of last year on methods for the determination of solids be repeated.

Approved.

FRUIT ACIDS.

It is recommended that the methods for the estimation of malic acid (active or inactive) be studied and that the Hartmann modification of the pentabromacetone method for citric acid¹ be studied with a view to its adoption as tentative.

Approved.

ASH IN FRUIT PRODUCTS.

It is recommended—

(1) That study of methods for the determination of the major bases (sodium, potash, calcium, magnesium) as well as of iron, aluminum, and manganese in plant ash be continued.

Approved.

(2) That the recommendation of last year for a study of the application to fruits of the procedure developed for chlorine in plant ash be repeated.

Approved.

CANNED FOODS.

It is recommended—

(1) That study be made of methods for the detection of spoilage in canned foods.

Approved.

(2) That the method given in the referee's report for estimating field corn in mixtures of field and sweet corn be adopted as tentative and that further study be discontinued (see p. 39).

Approved.

VINEGARS.

It is recommended that the recommendations approved last year² be continued.

¹ *This Journal*, 1928, 11: 257.

² *Ibid.*, 82.

MEAT AND MEAT PRODUCTS.

It is recommended—

(1) That the revised wording of the method for the determination of total nitrogen in meats adopted as official last year (first action)¹ be made official (final action).

Approved.

(2) That the method given by the referee for added water in sausage and similar meat products be adopted as tentative (see p. 43).

Approved.

SEPARATION OF MEAT PROTEINS.

It is recommended—

(1) That further work be done on the extraction of meat protein fractions and their separation by the method described in the associate referee's report.

Approved.

(2) That the Howe method² be compared with these methods.

Approved.

(3) That collaborative work be done when possible in connection with Recommendations (1) and (2).

Approved.

FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the steam distillation method for the determination of oils of lemon, orange, and lime in cottonseed oil and in mineral oil described in the report of the referee last year be adopted as official (final action)³.

Approved.

(2) That a study of the above method be continued with a view to extending it to other non-alcoholic flavors.

Approved.

(3) That the colorimetric method for the determination of small quantities of anthranilic acid ester described in the report of the referee last year be adopted as official (final action)⁴.

Approved.

(4) That the gravimetric method for the determination of large quantities of anthranilic acid ester described in the report of the referee last year be adopted as official (final action)⁵.

Approved.

(5) That the present official method for citral in lemon and orange extracts⁶ be dropped (first action) and that the method given in the referee's report be adopted as official (first action). (See p. 48.)

¹ *This Journal*, 1928, 11: 82.

² *J. Biol. Chem.*, 1924, 61: 493.

³ *This Journal*, 1928, 11: 45.

⁴ *Ibid.*, 46.

⁵ *Ibid.*, 47.

⁶ *Methods of Analysis*, A. O. A. C., 1925, 354.

The committee suggests that more extensive collaborative work be undertaken before final action.

Approved.

(6) That an effort be made to obtain a method for the determination of total aldehydes in lemon and orange extracts which the association will be warranted in adopting as an alternative official method.

Approved.

GELATIN.

It is recommended—

(1) That study of the preparation of sample be continued.

Approved.

(2) That study of methods for copper and zinc in gelatin be continued.

Approved.

CACAO PRODUCTS.

It is recommended that methods for the determination of milk solids and sucrose in cacao products be studied.

CRUDE FIBER.

It is recommended that the method for the determination of crude fiber proposed by the associate referee be further studied collaboratively, the study being extended to bitter, sweet, and milk cacao products.

Approved.

CACAO BUTTER.

It is recommended that study be continued on methods for the detection of foreign fats in cacao fat and in the fat of cacao products in general, and that special attention be given to quantitative aspects.

Approved.

CEREAL PRODUCTS.

FLOUR.

It is recommended—

(1) That the tentative method for sampling flour, adopted as official (first action) last year, be kept in its present status until further work has been done.

Approved.

(2) That the associate referee continue studies on rapid methods of ashing flour, giving special attention to the use of glycerol-alcohol mixture.

Approved.

(3) That the associate referee study the nature of the losses occurring when ash is fused.

Approved.

(4) That the F. A. C. method for the determination of unsaponifiable matter in fats and oils¹, as modified and adopted as tentative for flour², be retained as tentative.

The committee also recommends further study including collaborative work.

Approved.

(5) That the method for the determination of glutenin specified by the associate referee be adopted as tentative.

Approved.

(6) That study on the determination of hydrogen-ion concentration of flour be continued to include a comparison of the use of quinhydrone and antimony electrodes.

Approved.

(7) That the study of methods for the determination and evaluation of the diastatic value of flour be continued.

Approved.

(8) That further study be conducted on the Seidenberg method³ and modifications thereof for the determination of chlorine in bleached flour.

Approved.

(9) That further study be made of the tentative method (Rask)⁴ for the determination of starch in flour (bread and alimentary paste) and that the method be compared with the diastase method, as modified by Hartmann and Hillig⁵.

Approved.

(10) That before final action is taken on the recommendation made last year to adopt the factor 5.83 for converting nitrogen into protein in wheat, further consideration be given to the advisability of adopting this factor for scientific work only and retaining the old factor, 5.7, for all commercial transactions and regulatory work as proposed by the referee this year.

The committee, however, does not favor the adoption of two conversion factors. Pending final action on this subject the factor 5.7 remains official.

Approved.

(11) That the official method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol⁶ be dropped and the method as modified by the associate referee be made official (first action) and studied further.

Approved.

¹ *This Journal*, 1926, 9: 45, 1927, 10: 35.

² *Ibid.*, 1928, 11: 37.

³ *Ibid.*, 1925, 8: 678.

⁴ *Ibid.*, 1928, 11: 37.

⁵ *Ibid.*, 1926, 9: 482.

⁶ *Ibid.*, 40.

(12) That the Associate Referee on Bleaching of Flour be directed to study methods to detect the use of benzoyl peroxide.

Approved.

(13) That consideration be given by the appropriate associate referee to the study of certain foreign methods of analysis, especially those which are used by foreign governments in testing flour imported from this country.

Approved.

BAKED CEREAL PRODUCTS.

It is recommended—

(1) That the referee make further study of the tentative method for the sampling of bread¹, especial attention being paid to different types of bread.

Approved.

(2) That the tentative method for the determination of total solids in an entire loaf of bread¹ be made official (first action).

Approved.

(3) That the official method for the determination of fat (by acid hydrolysis) in flour² be adopted as tentative for baked cereal products and that this method be compared with the present tentative method³ for fat in baked cereal products.

Approved.

(4) That further study be made of the methods to determine lipoids in baked products.

Approved.

(5) That further work be done toward the development of methods for the determination of milk solids in bread.

Approved.

(6) That consideration be given to the development of methods for the estimation of rye flour in rye bread.

Approved.

(7) That the official method for the determination of chlorides in the ash of alimentary paste⁴ be made official for baked cereal products (first action).

Approved.

(8) That the official methods for moisture determination in flour⁵ be made official for air-dried baked cereal products (first action).

Approved.

¹ *This Journal*, 42.

² *Ibid.*, 1926, 9, 41.

³ *Methods of Analysis*, A. O. A. C., 1925, 231.

⁴ *Ibid.*, 1925, 232; *This Journal*, 1927, 10: 34.

⁵ *This Journal*, 1926, 9: 39.

(9) That the official method for the determination of crude fiber in flour¹ be made official for air-dried baked cereal products (first action).

Approved.

(10) That the official method for the determination of organic and ammoniacal nitrogen in alimentary paste² be made official for air-dried baked cereal products (first action).

Approved.

(11) That consideration be given to the study of baked products other than bread.

Approved.

(12) That the standard experimental baking test proposed by the associate referee³ (see p. 41) be adopted as tentative and that it be subjected to collaborative study.

Approved.

(13) That the associate referee make a recommendation next year on the subject of total solids in an entire loaf of bread by the 130° air oven and other rapid methods.

Approved.

(14) That further collaborative work be done with the tentative F. A. C. method for the determination of the unsaponifiable matter in the fats of baked cereal products.

Approved.

ALIMENTARY PASTE.

It is recommended—

(1) That the tentative method for collecting and preparing a sample of alimentary paste⁴ for analysis be further studied with the view to making it official.

Approved.

(2) That the methods now official for the determination of moisture in flour⁵ be adopted as official for alimentary paste (first action).

Approved.

(3) That further collaborative work be done with the tentative F. A. C. method for the determination of the unsaponifiable matter in the fats of alimentary paste (and baked cereal products).

Approved.

(4) That the tentative method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol³ be dropped and that the modification proposed by the associate referee be made official for alimentary pastes (first action). (See p. 40.)

Approved.

¹ *Methods of Analysis*, A. O. A. C., 1925, 225.

² *Methods of Analysis*, A. O. A. C., 1925, 232; *This Journal*, 1927, 10: 34.

³ *Cereal Chem.*, 1928, 5: 158.

⁴ *This Journal*, 1926, 9: 43.

⁵ *Ibid.*, 39-40.

(5) That the official method for the determination of crude fiber in flour¹ be made official for alimentary paste (first action).

Approved.

(6) That the associate referee make a recommendation next year on the subject of air-oven methods for the determination of total solids.

Approved.

(7) That a report on the tentative acid-hydrolysis method for fat and the tentative method for lipoids and lipid phosphoric acid (P_2O_5) in alimentary paste, recommended as official (first action) last year, be made by the referee next year.

Approved.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL².

The Crop Protection Institute was organized in 1920 under the auspices of the National Research Council. It has provided a neutral agency for the discovery, development, and testing of materials which may be useful in the protection of crops (plants and animals) from injury by insects and plant diseases and the extension and more efficient use of material for fertilization. The Institute has served as a medium through which various problems and products of American industries could be investigated; it has proved of mutual advantage to the industries and research agencies. The manner in which the Institute has handled its work has made possible a greater use of the highly developed facilities in existence at the Experiment Stations and other publicly supported research institutions and of the expert training and skill of their staffs.

Many of the investigations under the auspices of the Institute are of direct interest to the chemist and have grown out of the research conducted by chemists employed in industrial laboratories. Many of the investigations under way need the help of the chemist for their full and satisfactory prosecution. The members of this association have a common interest in the work of the Crop Protection Institute. A closer and greater cooperation on many problems would prove mutually beneficial.

During the year June 30, 1927-June 30, 1928, the treasury of the Crop Protection Institute handled more than \$75,000. Its receipts for that period amounted to over \$53,000 and its expenditures were more than \$45,000. It started its new year of work with \$30,000 in the bank.

This will give some idea of the growth of the work. In September, 1928, it had twenty-one men on the payroll for full or part-time work,

¹ *Methods of Analysis*, A. O. A. C., 1925, 225.

² Presented by R. B. Doerner.

the research projects being conducted in twelve universities or experiment stations, in addition to two projects at present at the Boyce-Thompson Institute.

Some of the studies now being pursued are in the field of seed-borne parasites, crown gall, cotton diseases, colloidal sulfur, pyrethrum, copper, and oil sprays, shale oils and their derivatives, furfuramid derivatives, and some special or patented products.

Most of these projects find their financial support from industrial companies.

The Institute publishes a monthly mimeographed circular and from time to time research bulletins and sends them to its members.

Its present Board of Governors is composed of W. C. O'Kane, P. J. Parrot, E. D. Ball, W. P. Flint, J. F. Adams, H. W. Anderson, I. E. Melhus, Burt Hartwell, H. J. Patterson. Paul Moore is Secretary-Treasurer.

H. J. PATTERSON.

Approved.

REPORT OF SECRETARY-TREASURER.

The following resignations were received during the year:

H. M. Lancaster, Referee on Maple Products.

J. O. Clarke, Referee on Vinegars.

F. C. Blanck, Referee on Cereal Products.

J. C. Munch, Associate Referee on Bioassay of Drugs.

A. M. Henry of the Food, Drug and Insecticide Administration, Philadelphia, Pa., was appointed in Clarke's place, and J. A. LeClerc, Bureau of Chemistry and Soils, replaced Blanck. B. L. Hartwell resigned as Representative on the Board of Governors of Crop Protection Institute of National Research Council. H. R. Watkins, U. S. Food, Drug and Insecticide Administration, represented this Association at the 7th annual meeting of the National Conference on Pharmaceutical Research.

Henry Clay White, one of the earlier active members of the association, passed away on December 1, 1927. An obituary by H. W. Wiley was published in the last number of *The Journal*, Vol. XI, No. 4, 1928. Philip Edward Chazal, one the the charter members, died on January 13, 1924, but notice of his passing was not brought to the attention of the secretary until recently. An obituary notice will be published in the next number of *The Journal*.

The following resolution, which was adopted at the last meeting of the American Association of Cereal Chemists, June 4, 1928, was forwarded to this association:

Be it further resolved that we extend greetings to the American Society of Bakery Engineers and to the Association of Official Agricultural Chemists; and that it is our desire to cooperate with them fully in all our mutual interests.

FINANCIAL REPORT ON PUBLICATIONS FROM

By W. W. SKINNER (Bureau of Chemistry and Soils,

RECEIPTS.

<i>Methods of Analysis.</i>		
Number	Price each	
22	\$5.50	\$121.00
329	5.00	1,645.00
82	4.40	360.80
213	4.00	852.00
		<u>\$2,978.80</u>
Plus gain on exchange		.05

Total	\$2,978.85
Minus re-deposited checks	10.00
	<u>\$2,968.85</u>

<i>Journal Subscriptions.</i>		
Number	Price each	
1	\$8.25	\$8.25
3	7.50	22.50
55	5.50	302.50
446	5.00	2,230.00
120	4.40	528.00
253	4.00	1,012.00
7	4.50	31.50
5	2.50	12.50
5	2.20	11.00
3	2.00	6.00
14	1.50	21.00
4	1.25	5.00
4	1.20	4.80
1	1.10	1.10
1	1.00	1.00
		<u>\$4,197.15</u>
Plus gain on exchange		.02

Total	4,197.17
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<i>Advertisements.</i>		
Number	Price each	
4	\$15.00	\$60.00
11	25.00	275.00
		<u>335.00</u>

<i>Miscellaneous.</i>		
Refund on stencils from P. L. Ricker		\$11.94
Revenue of Wiley Medallion Fund from C. A. Browne		10.00
Refund for registration cards and programs from Secretary-Treasurer's account		47.25
		<u>69.19</u>

<i>Reprints.</i>		
University of Arizona, Tuscon, Ariz.		\$5.06
A. R. Bliss, Memphis, Tenn.		10.63
R. L. McNeill, Philadelphia, Pa.		2.68
P. W. Morgan, Freeport, Ill.		2.15
H. O. Moraw, Indianapolis, Ind.		6.68
I. M. Williams, Madison, Wis.		4.96
A. Alfend, St. Louis, Mo.		4.75
Amer. Pharm. Mfg. Assn., New York City		.50
H. Runkle, Chicago, Ill.		5.02
H. W. Wiley, Washington, D. C.		5.89
M. J. Blish, Lincoln, Nebr.		7.07
Kentucky University, Lexington, Ky.		6.53
O. S. Rask, Baltimore, Md.		10.25
University of Tennessee, Memphis, Tenn.		12.84
C. A. Browne, Washington, D. C.		1.50
F. W. Zerban, New York City		12.78
D. H. Tilden, San Francisco, Calif.		6.88
J. N. Taylor, Washington, D. C.		2.59
Expt. Chemistry Department, Kingston, R. I.		8.25
O. B. Winter, E Lansing, Mich.		4.88
A. L. Prince, New Brunswick, N. J.		5.89
H. A. Schuette, Madison, Wis.		2.10
H. C. Waterman, Washington, D. C.		1.00
P. A. Sigler, Washington, D. C.		4.00
		<u>134.88</u>

Total	\$7,705.04
Total for Methods, Journal, Ads., Reprints and Miscellaneous	1,192.99
Bank Balance of October 1, 1927	

Total	\$8,898.03
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OCTOBER 1, 1927, TO OCTOBER 1, 1928.

Washington, D. C.), *Secretary-Treasurer*.

DISBURSEMENTS.

		Amount	Check No.
1927			
Oct. 12	Industrial Printing Co., bill of 8-20-27.....	\$1,088.01	229
Oct. 21	Estelle L. McCoy, mailing <i>Journal</i> and office expenses.....	50.00	230
Oct. 24	Postmaster, Washington, D. C., mailing <i>Journals</i>	25.00	231
Nov. 12	Industrial Printing Co., bill of 10-6-27.....	46.50	232
Nov. 26	E. P. Secker, Inc., bill of 11-26-27.....	11.69	233
Dec. 3	Industrial Printing Co., bill of 11-30-27.....	53.29	234
Dec. 3	Estelle L. McCoy, office expenses.....	50.00	235
Dec. 3	R. P. Andrews Co., bill of 11-22-27.....	2.73	236
Dec. 13	Colonial Printery, Inc., bill of 12-13-27.....	8.00	237
Dec. 13	Industrial Printing Co., bill of 12-8-27.....	12.65	238
Dec. 13	Industrial Printing Co., on account bill of 10-31-27.....	500.00	239
Dec. 20	R. P. Andrews, bill of 11-23-27.....	2.48	240
Dec. 21	Postmaster, Washington, D. C., box rent, quarter ending 3-31-28.....	2.00	241
Dec. 29	Industrial Printing Co., balance on bill of 10-31-27.....	655.97	242
1928			
Jan. 13	Central Fire Insurance Co., for storing <i>Methods</i>	4.20	243
Jan. 14	Industrial Printing Co., on account, bill of 6-30-27.....	500.00	244
Jan. 28	Industrial Printing Co., on account, bill of 6-30-27.....	500.00	245
Jan. 28	Estelle M. McCoy, office expenses.....	50.00	246
Jan. 31	J. M. Bartlett, back numbers of <i>Journal</i>	8.00	247
Feb. 10	G. C. Spencer, back numbers of <i>Journal</i>	1.00	248
Mar. 2	Industrial Printing Co., on account, bill of 6-30-27.....	500.00	249
Mar. 6	Estelle L. McCoy, office expenses.....	50.00	250
Mar. 23	Postmaster, Washington, D. C., box rent, quarter ending 6-30-28.....	2.00	251
Mar. 24	Industrial Printing Co., on account, bill of 6-30-27.....	234.88	252
Apr. 3	E. P. Secker, Inc., bill of 3-29-28.....	11.50	253
Apr. 6	Industrial Printing Co., bill of 3-31-28.....	56.50	254
May 1	Underwood & Underwood, picture of F. B. Power.....	3.00	255
May 2	Industrial Printing Co., balance on bill of 6-30-28.....	1,000.00	256
May 17	Cash, mailing <i>Journals</i> and office expenses.....	50.00	257
May 29	Postmaster, Washington, D. C., on account, stamped envelopes.....	19.30	258
June 4	Industrial Printing Co., bill of 2-29-28.....	1,094.49	259
June 4	Industrial Printing Co., bill of 5-17-28.....	90.40	260
June 16	University of Illinois, reimbursement for dues.....	5.00	261
June 25	Postmaster, Washington, D. C., box rent, quarter ending 9-30-28.....	2.00	262
June 25	Postmaster, Washington, D. C., balance on envelopes.....	100.00	263
July 2	J. J. Betton, bond for Mrs. McCoy.....	2.50	264
July 2	Industrial Printing Co., bill of 6-14-28.....	87.45	265
July 20	Cash, mailing and office expenses.....	50.00	266
Aug. 2	Industrial Printing Co., bill of 5-29-28.....	1,075.45	267
Aug. 2	Industrial Printing Co., Fire Insurance Policy Books.....	2.80	268
Aug. 3	F. W. Faxon, refund on <i>Journal</i>	4.00	269
Aug. 14	Industrial Printing Co., bill of 8-10-28 (see dues account). ..	5.75	270
Aug. 17	Industrial Printing Co., bill of 8-16-28 (see dues account). ..	41.50	271
Aug. 30	Cash, office expenses.....	50.00	272
	Plus bank balance, October 1, 1928.....	787.99	
	Total.....	\$8,898.03	

The Carnegie Institute of Technology invites this association to be represented at the Second International Conference on Bituminous Coal, to be held in Pittsburgh, November 19-24, 1928. It is not considered necessary to have the association represented at this meeting. Invitations were also received from the National Cathedral Association to visit Mt. St. Alban and from the Pan American Union to visit the Pan American Building. These invitations are extended to the individual members, but it is not possible to arrange official visits.

From the financial report it will be noted that receipts from subscriptions to *The Journal* and ads total \$4,197.17 and that the approximate cost of a volume is \$4,413.92, or a difference of approximately \$216.75 each year. It is evident that this yearly deficit could be met easily if it were possible to increase the subscription list to 1,000.

At the meeting of the Executive Committee held just prior to this meeting the treasurer's report was presented and fully considered. The treasurer was instructed to invest the accumulated surplus funds of the association as in his opinion seemed desirable. The report of the chairman of the Board of Editors was also considered. It was recommended that Mr. Deemer be permitted to decide where contributed papers may be published. The secretary presented a report on the progress made in revising the Wiley books.

This year 71 organizations paid membership dues compared with 62 last year. According to the available records, all the states except New Mexico have paid dues at some time. Twelve of the organizations are in arrears, leaving seven states unrepresented at the present time.

The financial report follows.

W. W. SKINNER.

FINANCIAL REPORT OF THE SECRETARY-TREASURER FROM OCTOBER 15, 1927, TO OCTOBER 1, 1928.

1927		RECEIPTS.		
Oct. 15	Bank balance		\$237.34	
	1927 dues received too late for inclusion in 1927 report,			
	6 at \$5.00		30.00	
	1928 dues from institutional members, 65 at \$5.00. . .		325.00	
				\$592.34
1927		DISBURSEMENTS.		
			Amount	Check No.
Oct. 20	Refund to Dr. Balcom for F. D. & I. Administration dues.		\$5.00	61
Oct. 21	Marian E. Lapp, 1927 meeting expenses.		25.00	62
Nov. 8	Bastian Bros. Co., bill of 10-22-27.		19.74	63
Nov. 8	Colonial Printery, Inc.		10.10	64
1928				
Feb. 20	Colonial Printery, Inc.		3.50	65
Feb. 29	Share for reprints of the Joint Meeting, 1926.		50.00	66
Mar. 28	Refund to <i>Journal</i> account, N. C. State College.		10.00	67
Aug. 20	Refund to <i>Journal</i> account for cards and programs, Industrial Printing Co., bills of 8-10-28 and 8-16-28.		47.25	68
			\$170.59	
Oct. 1	Bank balance		421.75	
	Total		\$592.34	

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

The committee begs to submit the following report on the proceedings of the Joint Committee on Definitions and Standards for the past year.

In connection with the presentation of last year's report reference was made to the passing of Julius Hortvet, who had for so many years rendered active and most valuable service as a member of this committee. Guy G. Frary, State Chemist of South Dakota, was appointed as Mr. Hortvet's successor on this subcommittee. At the December, 1927, meeting, owing to these and other changes in the personnel, the committee was reorganized with W. S. Frisbie as chairman.

Since the last convention three meetings of the Joint Committee have been held, one during the week of December 5, 1927, one in the week of April 16, 1928, and the third during the week of October 22, 1928. Because the usual fall meeting occurred just previous to this convention, three meetings are recorded this year instead of the usual two.

DECEMBER MEETING.

At the December meeting definitions and standards for tea and for sage were revised and adopted, and subsequently approved by the Secretary of Agriculture, these being promulgated February, 1928, as Supplement No. 1 to S. R. A. F. D. No. 2, the latter being the current designation of a new edition covering definitions and standards for food products as revised to December, 1927.

The revisions for tea and sage are as follows:

1. *Tea* is the tender leaves, leaf buds, and tender internodes of different varieties of *Thea sinensis* L., prepared and cured by recognized methods of manufacture. It conforms in variety and place of production to the name it bears; contains not less than four per cent (4%) nor more than seven per cent (7%) of ash; and meets the provisions of the act of Congress approved March 2, 1897, as amended, regulating the importation and inspection of tea.

47. *Sage* is the dried leaf of *Salvia officinalis* L. It contains not more than twelve per cent (12%) of stems (excluding petioles) and other foreign material.

At this meeting proposed definitions and standards for purified middlings, farina, and semolina were also tentatively adopted, but final action was deferred until the next meeting.

A conference on the composition of mayonnaise was held with several manufacturers. The matter of proper limits for oil and egg content and the propriety of the use of stabilizers received extensive consideration, and a proposed definition was tentatively adopted. Definitions for strained tomato products were completed, but action was deferred.

Definitions and standards for so-called process cheese were discussed, particularly in reference to the question of pasteurization. A proposed definition for sweet cream butter was considered, but this subject was

tabled. Further consideration was also given to a definition and standard for cream meal, but final action was postponed pending the revision of the entire schedule for cereal corn products.

Definitions for graham bread and for entire or whole wheat bread were discussed, but action was deferred pending the adoption of definitions for flours sold under these names.

APRIL MEETING.

At the April meeting the subject of process or emulsified cheese came up for renewed consideration. It was decided to so divide this topic as to recognize the two following classes of this product as appearing to be commercially existent: (1) blended, pasteurized products having a composition normal for cheese under the respective varieties, and (2) products of this general class but representing deviations from what has been generically defined and accepted as cheese, in that they have been produced by the use of chemical emulsifiers and of limited quantities of water. At this meeting analytical evidence was presented and accepted as corroborative of the claims of the manufacturers that the process employed is such as to result in a substantial reduction of the bacterial content. Definitions and standards covering these two classes of products were adopted and approved, being promulgated under date of August, 1928, as a part of Supplement No. 2 to the current revision. They provide as follows:

Pasteurized cheese, pasteurized-blended cheese, is the clean, sound, pasteurized product made by comminuting and mixing, with the aid of heat and water, one or more lots of cheese into a homogeneous, plastic mass. The name "Pasteurized cheese", "Pasteurized-blended cheese", unqualified, is understood to mean Pasteurized Cheddar cheese, Pasteurized-blended Cheddar cheese, and applies to a product which conforms to the standard for Cheddar cheese. Pasteurized cheese, Pasteurized-blended cheese, bearing a varietal name, is made from cheese of the variety indicated by the name and conforms to the limits for fat and moisture for cheese of that variety.

Emulsified cheese, "process cheese", is the modified cheese made by comminuting and mixing one or more lots of cheese into a homogeneous, plastic mass, with the aid of heat, with or without the addition of water, and with the incorporation of not more than 3 per cent of a suitable emulsifying agent. The name "emulsified cheese", "process cheese", unqualified, is understood to mean emulsified Cheddar cheese, process Cheddar cheese, and applies to a product which contains not more than 40 per cent of water and, in the water-free substance, not less than 50 per cent of milk fat. Emulsified cheese, process cheese, qualified by a varietal name, is made from cheese of the variety indicated by the name, and conforms to the limits for fat and moisture for cheese of that variety.

At this meeting two important and well attended hearings were held. That concerning definitions for purified middlings, semolina, and farina brought in representatives of the millers, the bakers, and the manufacturers of macaroni and allied products. One of the topics particularly provocative of discussion was the amount of flour that should be per-

mitted in alimentary pastes. Definitions and standards finally adopted and approved by the Secretary under date of June 27, 1928, are as follows:

12. *Purified middlings* is the clean, sound granular product obtained in the commercial process of milling wheat, and is that portion of the endosperm retained on 10 XX silk bolting cloth. It contains no more flour than is consistent with good commercial practice, nor more than fifteen per cent (15%) of moisture.

13. *Semolina* is the purified middlings of durum wheat.

14. *Farina* is the purified middlings of hard wheat other than durum.

The hearing on mayonnaise drew a large attendance of the manufacturers of this product, the makers of machines for the use of small local manufacturers, food inspection chemists, and others interested. Some of those present contended for a low oil content and a high egg content, while other prominent makers claimed that as mayonnaise is presumed to be essentially an oil product, a relatively high minimum oil limit should be fixed. Some of the manufacturers claimed that the use of upwards of one per cent of a stabilizing material is indispensable in commercial practice, while others maintained that this is not essential and should be looked upon as an adulterant. The committee obtained considerable valuable information, and it was also fortunate in having the benefit of an extensive series of analyses and an exhaustive presentation of the subject by H. G. Lepper, from the standpoint of the cook as well as that of the dealer. Final action upon the adoption of a definition and standard was deferred.

Revision of the definition and standards for ice cream, as originally promulgated many years ago, received some consideration at the meetings this year. Some time ago a tentative revision was adopted by the committee and put out to the trade following a number of hearings and an extensive consideration of the subject. Owing to the sharp dissension from this proposal on the part of the industry in regard to the fat standard named as well as to other stipulations, and to the present chaotic situation with respect to state fat standards, further progress in this matter has seemed almost impossible. This condition has been more pronounced because the committee considers that any action taken might be construed, more than ordinarily, to serve in place of legislation, or, in a sense, to usurp a legislative function, because the matter has already been the subject of special legislation by a large proportion of the states. Since the issuance of the tentative revision, one of the provisions of which is a minimum 12 per cent butter fat limit for plain ice cream, the Congress has enacted, in place of the original 14 per cent, a 10 per cent limit for the District of Columbia, and a number of the states have, in a contrary direction, revised their fat standards upward.

Another complication in the case of a product such as ice cream, is the desirability of the establishment of a sanitary requirement, such as

a stipulation calling for pasteurization and a maximum bacteria limit, but the committee is inclined to question whether, as a body functioning under the Food and Drugs Act, such a feature can properly come within the scope of its action. For the foregoing reasons, therefore, this topic remains in *status quo*.

OCTOBER MEETING.

During the meeting held this past week, considerable constructive work was accomplished. Under the spice schedule, the present definition and standard for cloves was reaffirmed. The matter of the revision of the ash content of mace was deferred pending the collection of more data. A definition and standard for mayonnaise received final adoption and has been transmitted to the Secretary of Agriculture for approval and promulgation.

Proposed definitions were adopted for wheat meal and graham meal and for whole wheat flour and entire wheat flour, and a tentative definition was also adopted to cover the product which has been erroneously referred to as "entire wheat flour". It is hoped that this action may serve to do away with the confusion that has long existed in this connection on the part of both the baking industry and consumers.

Revision in the form of final action was made concerning the oils and extracts of cassia and cinnamon in recognition of the fact that for a long time practically no Ceylon cinnamon oil has been employed in this country for food purposes, so far as known all flavoring extracts of this variety being based upon the type of oil known as cassia cinnamon.

The definitions for ginger ale and for ginger ale flavor were revised to recognize the use of caramel color as optional, and similar action was taken with respect to sarsaparilla. The definition for sarsaparilla flavor was also revised in other respects. It was agreed that definitions and standards for other carbonated beverages shall receive early attention on the part of the committee, with a view to extending this schedule materially.

The subjects of iodized salt and of a moisture standard for potato chips were briefly considered and action thereon with respect to the formulation of definitions and standards was postponed.

C. D. HOWARD.
E. M. BAILEY.
GUY G. FRARY.

Approved.

REPORT OF COMMITTEE ON SAMPLING.

No formal report was given by the Chairman of the Committee on Sampling. The following report on the subject of sampling in connection with fertilizers was presented by R. N. Brackett.

BIBLIOGRAPHY ON SAMPLING OF FERTILIZERS.

By R. N. BRACKETT.

At the 39th meeting of this association, 1923, the Executive Committee provided for the appointment of a new committee to study methods of sampling¹, and the following year the work of the committee was outlined².

In the second report made at the 41st annual convention, the committee again reported as follows: "The individual members, chosen by reason of their familiarity with the various fields of activity of this association, have agreed during the coming year to prepare a complete bibliography on experimental work and suggestions for the proper sampling of the various types of products".

Apparently the duty of the one charged with preparing a bibliography on sampling fertilizers is limited to the collecting of samples for analysis and is not concerned with the preparation of the samples in the laboratory for analysis.

The collecting of samples of fertilizers in regard to the method followed and the quantity taken had been provided for by the various states in their laws, or by the regulations of those charged with the inspection of fertilizers. This association concerned itself only with the preparation of the samples for analysis until the 33rd Annual Convention, 1916, when a committee was appointed "On Methods of Sampling Fertilizers to Cooperate with a Similar Committee of the American Chemical Society". This committee consisted of C. H. Jones, Chairman, W. J. Jones, Jr., Indiana, and B. F. Robertson, South Carolina.

Chairman Jones reported progress at the 34th Annual Convention, 1917³.

E. G. Proulx was appointed on the committee in place of W. J. Jones, Jr., deceased. The Committee had the cooperation of F. S. Lodge, Chairman of American Chemical Society committee, and of O. S. Roberts of Indiana.

At the 35th meeting, 1919 (there having been no meeting in 1918 on account of the Great War), the committee made a detailed report and included analyses of samples drawn after shipment.

RECOMMENDATIONS.

The following recommendations were presented:

- (1) That a sampler be used that removes a core from the bag from top to bottom.
- (2) That at least a pound of the material should constitute each official sample sent to headquarters.
- (3) That the entire sample submitted to the chemist be passed through a 10-mesh sieve previous to its subdivision for analysis.

¹ *This Journal*, 1924, 7: 291.

² *Ibid.*, 1925, 8: 287.

³ *Ibid.*, 1920, 4: 287.

(4) That cores shall be taken from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case a core shall be taken from 1 bag from each additional ton represented. If there are less than 100 bags, not less than 10 bags shall be sampled, provided that in lots of less than 10 bags all bags shall be sampled.

At the 36th meeting, 1920, Chairman Jones reported that no new recommendations would be presented to the association¹.

BIBLIOGRAPHY ON SAMPLING FERTILIZERS.

- (1) 1907. The Sampling and Sending of Fertilizers, etc., for Free Examination. Mass. Sta. Circ. 10, p. 3; Exp. Sta. Record, 1907, 19: 813.
- (2) 1910. Accuracy in Taking and Preparing Mixed Fertilizer Samples. F. B. Porter. *J. Ind. Eng. Chem.*, 1910, 2: 149.
- (3) 1912. A Note on Sampling. W. J. Sharwood. *J. Ind. Eng. Chem.*, 1912, 4: 227.
- (4) 1912. Sampling of Fertilizers. L. Grandeau. *J. agr. prat.*, 1905, 9: 533.
- (5) 1916. Better Samples from the Fertilizer Factory. A. J. Lawrence. *J. Ind. Eng. Chem.*, 1916, 8: 1145.
- (6) 1916. Note on the Results of Analyses of Fertilizer Samples Taken with Different Styles of Samplers. F. B. Carpenter. *J. Ind. Eng. Chem.*, 1916, 8: 1144; Exp. Sta. Record, 1917, 36: 711.
- (7) 1917. The Sampling of Fertilizers. F. S. Lodge. *J. Ind. Eng. Chem.*, 1917, 9: 167.
- (8) 1917. Report of Committee on Methods of Sampling Fertilizers to Cooperate with a Similar Committee of the American Chemical Society. *This Journal*, 1920, 4: 287, 594.
- (9) 1919. A Trial with Two Types of Fertilizer Samplers. L. D. Haigh. *This Journal*, 1921, 4: 597.
- (10) 1920. Report of Committee on Methods of Sampling Fertilizers to Cooperate with a Similar Committee of the American Chemical Society. *This Journal*, 1922, 5: 315.
- (11) 1922. Kellogg's Sampling Horn. J. W. Kellogg. *J. Ind. Eng. Chem.*, 1922, 14: 632.

The following paper on the Preparation of a Fertilizer Sample for Analysis was also prepared by R. N. Brackett.

PREPARATION OF A FERTILIZER SAMPLE FOR ANALYSIS.

By R. N. BRACKETT.

Although it appears that the Committee on Sampling is concerned only with a bibliography on sampling fertilizers to be sent to inspection laboratories, it may be of interest to give a resumé of the work relating to the drawing of sub-samples in the laboratory and their preparation for analysis as practiced by this association.

At the 1st Annual Convention of the Association of Official Agricultural Chemists, into which the Convention of Agricultural Chemists

¹ *This Journal*, 1922, 5: 315.

resolved itself at Philadelphia, September 9, 1884, the so-called Atlanta method of determining phosphoric acid was adopted. The paragraph with regard to sampling, certainly sufficiently liberal if not vague, reads as follows:

The sample should be well intermixed and properly prepared so that separate portions shall accurately represent the substance under examination, without loss or gain of moisture.

No change was made in the preparation of sample for analysis at the third convention held at Washington in 1886.

At the 4th Annual Convention, 1887¹, at Washington, Dr. McMurtrie presented the report of the committee to consider the communication of the Illinois State Board of Agriculture, as follows:

Your committee, to whom was referred the matter of the request of the State Board of Agriculture of Illinois, that the association adopt (1) a uniform series of blanks for use of all interested in inspection of commercial fertilizers, and (2) a clearly expressed definition of the term commercial fertilizer, have to report that it appears that in the conditions of the laws of the different States it is impracticable for this association to take action upon these points. Only methods for taking samples, making analyses, and stating results can be recommended with any satisfaction to all concerned.

In commenting on the report, Chazal said that the objection made, that such an inquiry was beyond the scope of the association, which had no power to compel State legislatures or boards of control to adopt its recommendations, would apply with equal force to the consideration of methods of analysis; that the association did not have any power to enforce the adoption by any State control of its methods of analysis—it only gives to certain methods the stamp of its approval and recommends their adoption for use; and that correct methods of sampling are certainly as essential as correct and uniform methods of analysis to the attainment of uniform results.

The paragraph on preparation of sample, however, remained as before, nor was there any change made at the 5th Annual Convention, at Washington, 1888².

At the 6th Annual Convention, at Washington, 1889³, Battle said that, in regard to the preparation of the sample, it was not stated through what sieve it should pass, that matter being left open to choice; a 20-mesh, a 60-mesh, or a 100-mesh sieve might be used. He also stated that in his own work he had found that a 20-mesh sieve would give a certain amount of citrate-insoluble phosphoric acid, a 40-mesh sieve less, a 60-mesh sieve still less, and a 100-mesh sieve the least of all, so that it seemed absolutely necessary that a standard size of sieve should be specified for use in the preparation of the sample; and that he used invariably a 20-mesh sieve.

¹ U. S. Dept. Agr. D v. Chem. Bull. 16.

² *Ibid.*, 19.

³ *Ibid.*, 24.

Mr. Frear said he had adopted the general plan of letting the manufacturer mix and grind his goods, and his analyst, who determined the total phosphoric acid, had given him results which agreed quite well with the samples he had himself analyzed of goods turned out upon the retail market; that, therefore, for a short time, he had not prepared his own samples at all before analysis. It was also stated that he had begun to hear from various quarters that his results were not altogether concordant with those of other chemists; that he had at once written to these gentlemen to ascertain what methods they used, and had found that, while there was some difference, the majority of them used sieves of from 20 to 25 mesh.

Jenkins' remarks were reported as follows: "With regard to the grinding, he felt no hesitation in grinding everything, and in his State fertilizer control work did grind everything that he analyzed, to pass through meshes the twenty-fifth of an inch in diameter. That was what he called an average sample. If it was not in that condition when he received it from the manufacturer, he made it so; then any one gram of it would represent 500 grams, or a ton, of the material, but this could not be unless the sample would pass through a one-twenty-fifth-inch-mesh sieve. He thought that unless there was great danger of losing moisture, it was well to follow the plan suggested by the New Jersey Station, mixing the material rapidly on paper each time before weighing out a sample for analysis".

Mr. Myers, the president, said that the particular question before the convention was as to the use of the sieve—whether the fertilizers should be sifted or not—the present discussion having arisen from the reading of Mr. Frear's paper, and that while speaking to the question it should be remembered that the more general subject before the association was as to whether the fertilizer should or should not be pulverized and sifted.

Other remarks made by Myers were reported as follows: "The habit of analyzing a fertilizer just as it came from the factory would inevitably get anybody who tried it into trouble; at least that had been his observation. If results were wanted that one could compare with others of his own and with those of his brother chemists, the fertilizer should be put through at least a 40-mesh sieve before the determination was begun, and should be well shaken, and the analyst should dip deep in taking out his sample, not using the outside. * * * He was confident that if the chemists could not get their samples well mixed, the results obtained would not be reliable; this was especially difficult in the case of bone meal. He thought that the association should prescribe some degree of fineness to which the fertilizer should be reduced before analysis".

The paragraph on "Preparation of Sample" read as follows:

The sample should be well intermixed and properly prepared and passed through a sieve having circular perforations one-twenty-fifth of an inch in diameter, so that

separate portions shall accurately represent the substance under examination, without loss or gain of moisture.

No changes were made at the 7th¹ and 8th² Annual Conventions.

At the 9th Annual Convention, 1892³, the paragraph on "Preparation of Sample" was changed to read as follows:

The sample should be well intermixed, finely ground, and passed through a sieve having circular perforations 1 mm. in diameter. The process of grinding and sifting should take place as rapidly as possible, so that there should be no loss or gain of moisture during the operation.

At the 10th Annual Convention, 1893⁴, Stillwell and Gladding presented a paper criticising the directions for preparing fertilizer samples, but they remained unchanged.

At the 11th Annual Convention, 1894⁵, after Mr. Huston had criticised the method, Mr. Van Slyke moved that, "in view of the crude condition in which many of the methods were, the president appoint a committee to go over the printed methods and arrange them in better shape, striking out such portions as are superfluous, and that this committee report next year". The motion was carried. No change, however, was made at the following meetings until the 33rd Annual Convention in 1916, when a Committee on Methods of Sampling Fertilizers, to Cooperate with a Similar Committee from the American Chemical Society, was appointed.

At the 34th Annual Convention, 1917, this committee recommended "that further study be made of the desirability of using a sieve having circular perforations $\frac{1}{2}$ mm. in diameter in place of the 1 mm. sieve now employed in the final preparations of the sample". The committee also reported as follows:

3. Care in subsampling in the laboratory.—It is suggested that the entire sample submitted to the chemist be passed through a 10-mesh sieve, thoroughly mixed and then subdivided by halving or quartering. The portion thus secured is then reduced to a suitable fineness for analysis. It has been found desirable to grind such samples as carry 4 per cent or more of ammonia sufficiently fine to pass through a sieve having circular perforations $\frac{1}{2}$ mm. in diameter.

At the 35th Annual Convention, 1919, the committee recommended—

(3) That the entire sample submitted to the chemist be passed through a 10-mesh sieve previous to its subdivision for analysis.

(4) That cores shall be taken from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case a core shall be taken from 1 bag from each additional ton represented. If there are less than 100 bags, no less than 10 bags shall be sampled, provided that in lots of less than 10 bags all bags shall be sampled.

¹ U. S. Dept. Agr. Div. Chem. Bull. 28.

² *Ibid.*, 31.

³ *Ibid.*, 35.

⁴ *Ibid.*, 38.

⁵ *Ibid.*, 43.

At the 36th Annual Convention, 1920, the committee made a final report renewing Recommendation (3), which was embodied as a suggestion in the first edition of *Methods of Analysis*, revised to November 1, 1919, making the paragraph on preparation of sample read as follows:

Reduce the gross sample by quartering to an amount sufficient for analytical purposes. Transfer to a sieve having circular openings 1/25 inch (1 mm.) in diameter, sift, breaking the lumps with a soft rubber pestle. Grind in a mortar the part remaining on the sieve until the particles will pass through. Mix thoroughly and preserve in tightly stoppered bottles. Grind and sift as rapidly as possible to avoid loss or gain of moisture during the operation.

It is recommended that the gross sample be taken by means of a sampler which removes a core from the top to the bottom of the bag; that at least a pound of the material shall constitute an official sample sent to the laboratory; and that the entire sample submitted to the chemist be passed through a 10-mesh sieve previous to its subdivision for analysis.

It will be noted also that the directions for preparing samples are made much more specific than they were in Bulletin 107.

Recommendations of the committee were embodied in the second (present) edition of *Methods of Analysis*, revised to July 1, 1924¹.

There was no work done or report made on "Preparation of Sample" at the meetings from 1921 until the second revision, and no change has been made since this revision.

BIBLIOGRAPHY ON PREPARATION OF FERTILIZER SAMPLE FOR ANALYSIS.

- (1) 1887. Report of Committee to Consider the Communication of the Illinois State Board of Agriculture, by William McMurtrie, and discussion by P. E. Chazal. U. S. Dept. Agr. Div. Chem. Bull. 16, p. 41.
- (2) 1889. Discussion of Preparation of Sample for Analysis, by H. B. Battle, William Frear, and E. H. Jenkins. U. S. Dept. Agr. Div. Chem. Bull. 24, p. 52.
- (3) 1893. Notes on the Preparation of Fertilizer Samples for Analysis, by Stillwell and Gladding. U. S. Dept. Agr. Div. Chem. Bull. 38, p. 19.
- (4) 1894. Discussion of Preparation of Sample for Analysis, by H. A. Huston and L. L. Van Slyke. U. S. Dept. Agr. Div. Chem. Bull. 43, p. 147.
- (5) 1917. Report of Committee on Methods of Sampling Fertilizers to Cooperate with a Similar Committee of the American Chemical Society. *This Journal*, 1920, 4: 287.

No report was made by the Chairman of the Committee on Bibliography.

¹ *Methods of Analysis*, A. O. A. C., 1925, 1.

REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of *The Journal and Methods of Analysis*, covering the period from October 1, 1927, to October 1, 1928, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 15, 1927, to October 1, 1928, and found the same to be correct as reported.

J. W. SALE.

W. W. RANDALL.

G. S. JAMIESON.

Approved.

REPORT OF COMMITTEE ON NECROLOGY.

During the past twelve months the committee has learned of the death of two chemists, each of whom occupied a prominent place in its personnel during its early years.

H. C. White, one of the founders of the association, died December 1, 1927. Since a full and sympathetic account of his life and work has been prepared by our honorary president and published in the latest issue of *The Journal*, the committee feels that it would be quite superfluous to introduce here any expression of its admiration of his character and its appreciation of his services.

The fact of the death of P. C. Chazal was not until recently brought to the attention of the committee. A study of his career is now being prepared for publication in *The Journal*; consequently the details of his life and work will not be entered into in this brief report. Suffice it to say that Dr. Chazal was president of the association in 1888.

It is not perhaps realized by the rank and file of the association that membership automatically begins with connection with an official laboratory and ends with the cessation of such connection. As a consequence, one who may have been active and prominent in its work may suddenly cease, through withdrawal from an official position, to have any continuing part in its life. His name disappears from the list of members and, in a comparatively short time, he may become a person quite unknown to the great mass of "official agricultural chemists".

The Committee on Necrology urges upon all members of the association its wish to be informed of the death of any member, or former member, as soon as it occurs, in order that proper action may be taken.

W. W. RANDALL.

C. A. BROWNE.

H. C. LYTHGOE.

Approved.

REPORT OF NOMINATING COMMITTEE.

The Nominating Committee desires to place in nomination the following names:

President: H. B. McDonnell, Agricultural Experiment Station, College Park, Md.

Vice-President: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

L. D. Haigh, Columbia, Mo.

F. C. Blanck, Washington, D. C.

J. W. Kellogg, Harrisburg, Pa.

Ex-Officio Member of Executive Committee:

Oswald Schreiner, Bureau of Chemistry and Soils, Washington, D. C.

F. P. VEITCH.

B. B. ROSS.

H. H. HANSON.

Approved.

It was moved, seconded, and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

REPORT OF COMMITTEE ON RESOLUTIONS.

(1) *Resolved:* That this association again thanks its honorary president for his greeting, extends to him its congratulations upon his manifest good health and spirits, and expresses the hope that he may long continue to be its living inspiration as he was so long its efficient guide.

(2) *Resolved:* That the retiring president deserves gratitude and praise for the manner in which the business of this convention has been conducted under his leadership.

(3) *Resolved:* That the thanks of the association are in special measure due to the secretary-treasurer and to his assistants for the labor and forethought which have ensured the success of this meeting.

(4) *Resolved:* That the members of the association wish to express their appreciation of the pains and skill that have been devoted by the Board of Editors, Miss Lapp, and their assistants to secure for *The Journal* that reputation for scholarly accuracy it so widely enjoys.

(5) *Resolved:* That the association herewith expresses its gratitude to Dr. A. W. Woods, the Director of Scientific Research, U. S. Department of Agriculture, for his courtesy in attending its session and addressing its members.

(6) *Resolved:* That this association regrets to learn of the serious illness of Dr. H. J. Patterson, and rejoices that the most recent reports indicate that his recovery will be prompt and complete.

(7) *Resolved:* That the management of the Hotel Raleigh has once more exhibited a care for the comfort of the members of the association and a concern for the success of the annual meeting which call for hearty thanks.

W. W. RANDALL.

C. A. BROWNE.

H. C. LYTHGOE.

Approved.

The convention continued in session through the noon hour and adjourned at 2:30 p. m. when all the business had been completed.

CONTRIBUTED PAPERS.

THE VERIFICATION OF THE 100° POINT OF THE VENTZKE SUGAR SCALE.

Introduction.

By C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.).

Since the time of the adoption of the present normal weight of 26 grams for instruments equipped with the German or Ventzke sugar scale by the International Commission upon Uniform Methods of Sugar Analysis in 1900, there has prevailed a considerable degree of uncertainty as to the accuracy of this value.

In 1905 Rolfe¹ called attention to the fact that the old Mohr cc. normal weight of 26.048 grams when recalculated to a basis of 100 true cc., or ml., at 20°C. was 26.01 grams and that the decision of the International Commission to use the even value 26.00 grams, involving an error of 0.04 per cent, was hardly in accord with their recommendation to weigh all samples to 0.001 gram or to 0.004 per cent. It seems very probable that the manufacturers of German scale saccharimeters adhered to the old rotation value of the 100° point of their scales and made no change when the new normal weight of 26.00 grams was adopted. It could thus come about that any inherent minus error in the old Ventzke scale was further increased by the omission of the slight fraction of approximately 0.01 gram in the new recalculated normal weight.

In 1912 at the New York meeting of the International Commission upon Uniform Methods of Sugar Analysis, Bates announced that preliminary investigations conducted at the Bureau of Standards indicated that the new normal weight of 26.000 grams of chemically pure sucrose gave a reading upon German scale saccharimeters which was 0.1° too low. A committee was therefore appointed by the International Commission to investigate the accuracy of the new normal weight, but the outbreak of the World War and the disturbed conditions which ensued thereafter have prevented a renewal of the meetings of the Commission and further international consideration of the subject.

In 1916 Bates and Jackson, of the Bureau of Standards², published a detailed account of their experiments, from which they concluded that the polarization of 26.000 grams of sucrose upon German scale saccharimeters, under the prescribed conditions, was 99.895°. This new value was adopted shortly thereafter by the Bureau of Standards in the standardization of quartz control plates and saccharimeters.

¹ The Polariscopes, p. 46.

² Bur. Standards Scientific Paper 268, 1926.

At the 1919 meeting of the Association of Official Agricultural Chemists, A. H. Bryan¹, who was Associate Referee on Sugar, made the recommendation "that when quartz plates are sent to the Bureau of Standards for certification they be requested to certify to the old value of the 100° point and not their new one—at least until this new value has been agreed to internationally". As a result of Bryan's report the present Committee of the A. O. A. C. on Quartz Plate Standardization and Normal Weight, consisting of Frederick Bates (Chairman), C. A. Browne and F. W. Zerban, was appointed by President Lythgoe at the 1919 meeting.

In 1921 Stanek², of the Czechoslovakian Sugar Experiment Station at Prague, published the results of an investigation in which he showed the polarization of 26.000 grams of pure sucrose (purified by a double precipitation with alcohol) upon German scale saccharimeters to vary from 99.81 to 99.90.

In 1924 Kraisy and Traegel³ of the German Institute for the Sugar Industry published the results of their extensive investigations upon the subject and announced the conclusion that the reading of 26.000 grams of pure sucrose, upon saccharimeters equipped with the Ventzke or German sugar scale, was 99.834°.

In view of the unfortunate lack of agreement upon the question, a series of cooperative experiments was initiated in 1926, at the request of the writer, who was at that time Chief of the Bureau of Chemistry, by the Carbohydrate Division of the Bureau of Chemistry in Washington and the New York Sugar Trade Laboratory in New York City, in order to determine if confirmation could be obtained upon any of the results previously reported. A preliminary report of this cooperative investigation was presented by Browne and Zerban⁴ at the 1927 meeting of the association. The accompanying papers by Balch and Hill of the Carbohydrate Division of the U. S. Bureau of Chemistry and Soils, and by Zerban, Gamble and Hardin of the New York Sugar Trade Laboratory, Inc., give the final corrected results of this investigation. They were presented at the recent September, 1928, meeting of the Sugar Division of the American Chemical Society at Swampscott, Mass., and are published through courtesy of *Industrial and Engineering Chemistry*.

The results of the present collaborative investigations confirm completely the previously published results of Bates and Jackson of the Bureau of Standards, and it was upon the basis of this agreement that the report of the association's Committee on Quartz Plate Standardization and Normal Weight, presented at the recent 1928 meeting, was made.

¹ *This Journal*, 1921, 4: 326.

² *Z. Zuckerind. oechoslovak. Rep.*, 1921, 45: 417, 425.

³ *Z. Ver. deut. Zuckerind.*, 1924, 74: 193.

⁴ *This Journal*, 1928, 11: 55.

Part I.

By R. T. BALCH and H. G. HILL (Carbohydrate Division, Bureau of Chemistry and Soils, Washington, D. C.).

In this reinvestigation of the 100° point of the Ventzke sugar scale it was decided to take exceptional care in the preparation of the sugar and to use specially constructed equipment, with the exception of the saccharimeter, in making the determinations.

PREPARATION OF SUCROSE.

The highest grade of commercial cane sugar was used as the raw material in the preparation of the recrystallized sucrose. A 50 per cent solution of the sugar in distilled water was treated with a generous amount of a vegetable carbon and filtered through a compactly woven paper filter precoated with a small quantity of kieselguhr. Some doubt was felt at first as to the feasibility of such a treatment, owing to the danger of contamination of the sugar solution by sugar-soluble substances from the carbon or kieselguhr. This fear was found unwarranted, however, as indicated by a preliminary analysis of the recrystallized sugar. Such a treatment very effectively eliminates the mineral constituents and probably whatever colloidal material may be present, which otherwise would have been removed only by several recrystallizations. Traces of reducing sugars are eliminated only by recrystallization, but by taking precautions to evaporate the solution at the lowest possible temperature and keeping the solution slightly alkaline to prevent undue hydrolysis of the sucrose, this impurity was satisfactorily removed with two recrystallizations. Some investigators have recommended the addition of sodium carbonate to maintain an alkaline condition, but owing to the fact that this substance would increase the ash content of the mother liquor, it is not so desirable, in the writers' estimation, as ammonia which, if present in the recrystallized sugar, is entirely eliminated by spontaneous evaporation. Since the quantity of concentrated ammonia necessary to produce an alkaline reaction in the sugar solution is only a few drops per liter, there need be little fear of contamination from this source.

The clarified sugar solution was refiltered through a thin pad of asbestos fiber while being drawn into the 3 liter distilling flask in which the concentration was conducted under a pressure of approximately 25 mm. at a temperature below 35°C. The sirup, after concentration to approximately 78 per cent solids content, was transferred to a glass precipitating jar, seeded with recrystallized sucrose, and stirred at frequent intervals until the greater part of the crystallization was completed, which process usually required approximately 2 hours. To insure a

complete crystallization, the massecuite was allowed to stand several hours longer before centrifuging. Separation of the crystals from the mother liquor was accomplished in a centrifuge fitted with a bronze basket lined with copper gauze, over which was placed a layer of fine-mesh silk bolting cloth. This simple and inexpensive arrangement was entirely satisfactory for retaining the fine sucrose crystals and no contamination of the sugar could be detected by analysis. After washing with sufficient acid-free alcohol (70-95 per cent) to free the crystals of a greater portion of the mother liquor, the sugar was removed from the basket and treated with an additional and sufficient quantity of 95 per cent alcohol to obtain a flowing mixture, and again centrifuged. As the impurity most difficult to remove from the sucrose was reducing sugars, this extra treatment with alcohol made possible a more complete removal of the mother liquor than was obtained simply by washing the crystals in the centrifuge. Even after the first crystallization the sucrose was in a very high state of purity. However, since it was not possible to obtain a satisfactory grade on each of the several crops of sugar from the original mother liquor, a second crystallization of the whole was made. The mother liquor from the second crystallization was worked back into the first liquor so that a minimum of waste occurred, and that from the several crops of sucrose comprising the first crystallization was discarded only when the sugar crystallized therefrom showed a distinct test for reducing sugars.

The recrystallized sucrose, after thorough washing with alcohol, was transferred to shallow enameled trays, covered with a large sheet of filter paper, and allowed to dry spontaneously for several days. After this preliminary drying the lumps were crushed and the sugar was passed through a 40-mesh phosphor-bronze screen to insure a homogeneous product. The sucrose was then transferred to clean, dry bottles, sealed, and placed in the dark at a temperature slightly above 0°C. until ready for use. The quantity of purified sucrose comprising the first lot was sufficient for conducting the first part of the investigation reported herein as well as for the tests conducted by The New York Sugar Trade Laboratory, Inc. For the second part of the investigation conducted by the writers, two additional lots of sugar were prepared; for one lot the procedure already described was followed, while the other was precipitated from the clarified and partially concentrated solution by means of neutral 95 per cent alcohol. Precipitation with alcohol was the method generally followed by the European investigators in preparing their pure sucrose and it was the object of the writers to determine whether the mode of preparing the sugar would have any bearing upon the results.

ANALYSIS OF THE SUCROSE.

The purity of the sucrose was determined by estimating the ash, reducing sugar, and moisture content.

The ash was determined on 10 gram samples by direct incineration in a platinum crucible at a relatively low heat.

In the method prescribed by Bates and Jackson¹, a modified Soldaini's reagent was used in estimating the reducing sugars. Following their procedure, there was obtained 1.2 mg. of cuprous oxide in contrast with their result of 1.1 mg. for purified sucrose. They concluded, after considerable study, that a greater portion of this reduced copper was caused by the sucrose itself and that the sugar contained probably less than 0.001 per cent of reducing sugars. From experiments conducted by one of the writers, in which known quantities of dextrose were added to pure sucrose, it was concluded that the purified sucrose contained reducing sugars equivalent to approximately 0.0015 per cent, it being assumed that these substances would probably have a slightly lower reducing power than dextrose.

Before subjecting the sucrose to polarimetric tests, it was further dried in a vacuum desiccator over phosphorus pentoxide. The moisture remaining in the sugar was determined at infrequent intervals during the course of the work by heating 10 gram samples in a vacuum oven for several hours at a temperature of 50°C. and approximately 25 mm. absolute pressure.

The averaged results of the analyses of the three lots of sugar are given in Table 1.

TABLE 1.
Analysis of purified sucrose.

LOT NO.	ASH <i>per cent</i>	MOISTURE <i>per cent</i>	REDUCING SUGARS <i>per cent</i>	TOTAL IMPURITIES <i>per cent</i>	CRYSTALLIZED FROM—
1	0.0015	0.0020	0.0015	0.005	water
2	0.0010	0.0015	0.0015	0.004	alcohol
3	0.0015	0.0000	0.0015	0.003	water

DESCRIPTION OF EQUIPMENT.

Flasks.—The flasks used were constructed especially for this work. The design was similar to that used by Bates and Jackson²; the exception was that only one graduation was placed upon the neck of the flask, that being at the 100 cc. capacity graduated at 20°C. After graduation these flasks were very carefully standardized with water. The necks of the flasks were 6–8 mm. in diameter; they terminated above the graduating mark in a bulb of approximately 25 cc. capacity and a ground

¹ Bur. Standards Scientific Paper 268, 1916, pp. 81–82.

² *Ibid.*, 1916, p. 97.

glass stopper. This bulb is an essential feature in obtaining a thorough mixing of the contents of the flask.

Weights.—The set of weights used in this investigation was recently standardized, and the necessary corrections were applied to the observed weighings.

Polariscope Tube and Thermometer.—The description of this equipment is included in the report of the New York Sugar Trade Laboratory, Inc., and will not be repeated here.

Saccharimeters.—A Frič saccharimeter (Bates model) was used for a greater portion of the observations, while a few were made on two separate Schmidt and Haensch instruments. The existing 100° points of these saccharimeters were very carefully checked against a recently standardized quartz control plate reading 99.859°.

The saccharimeters were placed in a thermo-regulated dark room maintained at $20^{\circ}\text{C.} \pm 1^{\circ}$, and illuminated by means of an electric "stereopticon" lamp of high intensity placed outside the room. Protection against body temperature which might affect the contents of the polariscope tube and quartz wedges was obtained by suitable screening. A potassium bichromate solution was used in the customary manner as a light filter.

PROCEDURE.

For the first 20 determinations, the data of which are compiled in Table 2, slightly more (about 0.005 gram) than 26 grams of the purified sucrose was roughly weighed into a nickel capsule, and quickly transferred to one of the special 100 cc. volumetric flasks that had been previously weighed. The flask was stoppered, wiped, and placed in the balance case for about 45 minutes before reweighing. Freshly boiled distilled water was then added to nearly the neck of the flask, the sugar was dissolved, and the flask was placed under vacuum a few minutes to remove entrapped air. The contents of the flask were mixed as thoroughly as possible before adding the last few cc. of water necessary to fill to the mark. The solution was cooled to exactly 20°C. , and any slight excess of water was evaporated by means of a gentle current of filtered air. This procedure also served for drying the inside of the neck of the flask of any possible moisture that might have adhered to it, thus avoiding the need for drying with filter paper, which would have proved much more troublesome. The flask, after the contents were carefully adjusted at 20°C. , was wiped dry, stoppered and placed in the balance case about 1 hour before weighing. All weighings were made on both left and right pans of the balance, and the results were averaged after correcting for the slight errors present in the weights. After thoroughly mixing the contents of the flask, the adjustable polariscope tube was filled, set at 199.0 or 201.0 mm., and allowed to stand in the polariscope

trough about 1 hour before reading in order to allow the solution to acquire exact room temperature. To avoid evaporation the tube was kept tightly stoppered. Ten readings of the saccharimeter were observed at each of the following lengths of the polariscope tube:

201.0	200.5	200.0	199.5	199.0
199.0	199.5	200.0	200.5	201.0

making a total of 100 readings for each sample investigated. The averaged results give the reading for a 200 mm. tube.

The readings on the quartz plate and zero point were read before and after the observations on the sucrose solution. The average of these zero points was used in correcting the polarization of the sugar solutions.

TABLE 2.

Data on solutions containing approximately old normal weight of sucrose.

DETER- MINATION NO.	WEIGHT OF SUGAR IN AIR	WEIGHT OF SUGAR IN VACUUM	WEIGHT OF SOLUTION IN AIR	WEIGHT OF SOLUTION IN VACUUM	STRENGTH OF SOLUTION (FROM WEIGHT IN VACUUM)	POLARIZA- TION (200 MM. AT 20°C.)
					per cent	
1	25.9978	26.0137	109.6711	109.7759	23.6972	99.802
2	25.9969	26.0128	109.6487	109.7534	23.7011	99.869
3	25.9893	26.0052	109.6424	109.7471	23.6956	99.897
4	25.9993	26.0152	109.6576	109.7616	23.7015	99.924
5	26.0225	26.0384	109.6723	109.7770	23.7194	99.924
6	25.9951	26.0110	109.6606	109.7653	23.6969	99.910*
7	25.9850	26.0009	109.6510	109.7557	23.6898	99.923
8	25.9938	26.0097	109.6624	109.7671	23.6954	99.874*
9	25.9961	26.0120	109.6558	109.7605	23.6989	99.891
10	26.0022	26.0181	109.6563	109.7610	23.7043	99.885
11	25.9910	26.0069	109.6535	109.7582	23.6947	99.889*
12	26.0017	26.0176	109.6635	109.7682	23.7023	99.912
13	25.9955	26.0114	109.6577	109.7624	23.6979	99.876
14	26.0023	26.0182	109.6593	109.7640	23.7038	99.917
15	25.9952	26.0111	109.6570	109.7617	23.6978	99.838†
16	26.0013	26.0172	109.6542	109.7589	23.7040	99.842†
17†	26.0147	26.0306	109.6674	109.7721	23.7133	99.916
18†	26.0247	26.0406	109.6618	109.7664	23.7237	99.889
19†	25.9987	26.0146	109.6605	109.7652	23.7002	99.933
20†	25.9978	26.0137	109.6934	109.7981	23.6923	99.909
Average	26.0000	26.0159	109.6603	109.7650	23.7015	99.902
Polarization of 26 grams corrected for impurities.....						99.907 ± 0.003

* Average of observers.

† Not included in average.

‡ The average of four determinations by Bates instrument gave 99.918.

The average of four determinations by S. & H. No. 5924 instrument gave 99.925.

The average of four determinations by S. & H. No. 5050 instrument gave 99.926.

The average temperature over all observations was 20.025°C.

In the calculation of the rotation of a normal weight (26 grams) of sugar the following corrections were included: (1) Tube length, (2) volume, determined from weights in vacuum in conjunction with specific gravity according to Plato, and (3) temperature of the solution being polarized. The correction for impurities was made only on the average polarization.

TABLE 3.
Data on solutions containing new normal weight of sucrose—26.026 grams per 100 cc.

DETER- MINATION NO.	SUGAR CRYSTALLIZED FROM—	WEIGHT OF SUGAR IN AIR grams	WEIGHT OF SUGAR IN VACUUM grams	WEIGHT OF SOLUTION IN VACUUM grams	WEIGHT OF SOLUTION IN VACUUM grams	STRENGTH OF SOLUTION (FROM WEIGHT IN VACUUM) per cent	POLARIZATION (CORRECTED) °V. (20°C.)	SPECIFIC GRAVITY 20°/20°	
								Assuming flask filled to calibrated capacity	Calculated from per cent sugar in vacuum according to Pinto's tables
21	Alcohol	26.0260	26.0419	109.6588	109.7634	23.7255	99.991	1.09957	1.09966
22	"	"	"	109.6655	109.7701	23.7240	99.999	1.09946	1.09965
23	"	"	"	109.8626*	109.9675	23.6815†	99.966†		
24	"	"	"	109.6498	109.7544	23.7274	99.995	1.09949	1.09967
25	"	"	"	109.6746	109.7793	23.7220	100.015	1.09975	1.09964
Average polarization—corrected for 0.004 per cent impurities								99.999	
26	Water	26.0260	26.0419	109.6587	109.7633	23.7255	99.967	1.09957	1.09966
27	"	"	"	109.6585	109.7631	23.7256	100.030	1.09957	1.09966
28	"	"	"	109.6604	109.7650	23.7251	100.010	1.09960	1.09966
29	"	"	"	Not weighed			100.017		
30	"	"	"	109.6664	109.7710	23.7238	99.987	1.09965	1.09965
31	"	"	"	109.6629	109.7675	23.7246	100.021	1.09963	1.09965
32	"	"	"	109.6656	109.7702	23.7240	99.976	1.09965	1.09965
33	"	"	"	109.6717	109.7763	23.7227	100.003	1.09970	1.09964
34	"	"	"	109.6625	109.7671	23.7247	99.996	1.09963	1.09965
35	"	"	"	109.6655	109.7701	23.7240	99.980	1.09964	1.09965
36	"	"	"	109.6634	109.7680	23.7245	100.035	1.09962	1.09965
37	"	"	"	109.6672	109.7718	23.7237	99.965	1.09966	1.09965
38	"	"	"	109.6685	109.7731	23.7234	100.004	1.09969	1.09965
39	"	"	"	109.6717	109.7763	23.7227	100.012	1.09970	1.09964
40	"	"	"	109.6665	109.7711	23.7238	100.009	1.09965	1.09965
Average polarization—corrected for 0.003 per cent impurities								100.004	
Grand averages								1.09964	1.09965

* Water added exceeded the calibrated mark.

† Not included in average.

‡ Corrected for volume.

For the second part of the investigation exactly 26.026 grams—the new normal weight of sugar calculated by Browne and Zerban¹ from the data compiled by Zerban, Balch and Hill, and by Bates and Jackson—was weighed out, made up to 100 cc., and polarized at 20°C., the procedure described above being followed. As a matter of record, the specific gravity of this solution was calculated from the weight in vacuo, it being assumed that the flask was filled to the calibrated capacity. The agreement between this value and Plato's table indicates the accuracy of this method. In Table 3 are given the results of the observations on the new normal weight solution.

DISCUSSION OF RESULTS.

Although the data as presented are practically self-explanatory, perhaps it will be advisable to point out a few interesting facts regarding them.

After applying all corrections the polarization of 26 grams of sucrose in 100 cc. gave an average value of $99.907^\circ \pm 0.003^\circ$ (calculated by the law of least squares). This value is very close to that determined by Bates and Jackson, namely 99.895° .

In Table 4 are recorded readings of the standard quartz plate used in this investigation. These data are given to indicate the variation from day to day that might be termed the personal factor. It is interesting to note the agreement between the averaged, observed and calibrated values, which indicates the correctness of the saccharimeter scale at this point. In order to relieve the observer from making the many observations required in the compilation of the data given in Table 3, it should be stated that the control plate was used exclusively in determining the zero readings in all the determinations recorded in this table.

Table 3 gives the data on sucrose solutions containing exactly 26.026 grams per 100 cc., this quantity being calculated from the rounded polarization of 26 grams per 100 cc. (99.90°) found by Balch and Hill, by Zerban, and by Bates and Jackson. The average polarization was found to be 100.003° , or in terms of 26 grams 99.903° , which agrees well with the previous determination. The other constants of this solution are the following: sugar by weight = 23.724 per cent, specific gravity = 1.09965.

SUMMARY.

1. The polarization of a solution containing 26 grams of sucrose per 100 cc. at 20°C. was found to be 99.907° Ventzke, which agrees well with the value determined by Bates and Jackson of the Bureau of Standards, and which disagrees with the values found by European investigators.

¹ *This Journal*, 1928, 11: 55.

2. The accuracy of this value was substantiated by determining the polarization of a solution containing 26.026 grams per 100 cc. at 20°C. The average value for the new normal weight solution was found to be 100.003° Ventzke. This solution contained 23.724 per cent sucrose and had a specific gravity of 1.09965.

TABLE 4.
Quartz plate readings.

DETERMINATION NO.	BEFORE READING SUCROSE SOLUTION	AFTER READING SUCROSE SOLUTION
1	99.871	99.874
2	99.870	99.882
3	99.887	99.844
4	99.877	99.886
5	99.889	99.871
6	99.840
7	99.855	99.861
8	99.851
9	99.843	99.840
10	99.859	99.841
11	99.857
12	99.831	99.828
13	99.867
14	99.864	99.866
15	99.869
16	99.845	99.841
17	99.837	99.837
18	99.888	99.854
19	99.837
20	99.851	99.846
Average	99.861	99.854
True total average.....		99.858
Calibration by U. S. Bureau of Standards .		99.859

Part II.

By F. W. ZERBAN, C. A. GAMBLE and G. H. HARDIN (New York Sugar Trade Laboratory, Inc., New York, N. Y.).

It was suggested by C. A. Browne that besides using varying weights of sucrose in the neighborhood of 26 grams, it would be advisable to vary the length of the solution. This procedure would further guard the observer from being influenced in his measurements by expected readings. The relation between rotation and tube length is strictly a straight-line function. This is not quite true for the relation between rotation and concentration, but the deviation from the straight line within the concentration range generally used in this type of investigation falls within the limit of experimental error.

The polariscope tube used in this work was designed by one of the writers in cooperation with C. W. Keuffel, of Keuffel and Esser Co., Hoboken, N. J., which firm furnished one of these tubes to each of the two institutions in which the investigation was carried out.

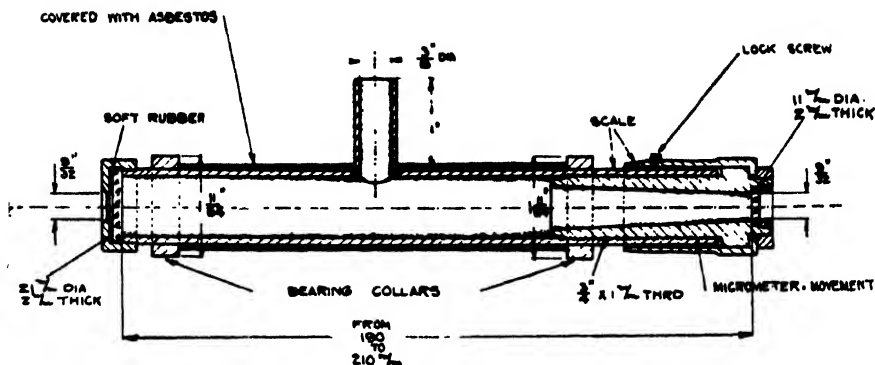


FIG. 1. POLARIZATION TUBE OF ADJUSTABLE LENGTH AND WITH MICROMETER SCREW.

The tube is shown in Figure 1. It is made of heavy brass tubing, 21.5 mm. outside diameter. Near each end there is mounted a brass flange having the same outside diameter as the standard tube heads, to fit into the trough of the regular saccharimeter with the Ventzke scale. One end of the tube is finished as in the usual polariscope tubes. An accurately machined female thread of exactly 1 mm. pitch is cut into the opposite end of the tube, and into this is screwed the other tube head, which is fitted closely over the tube proper and provided with the usual cover glass and screw cap. By turning a milled head, this end of the tube can be moved back and forth; in this way the distance between the inside planes of the cover glasses can be controlled within the limits of 190 and 210 mm. The length of the column of solution is read on a millimeter scale engraved on the upper side of the tube itself. At the end nearest this scale, the periphery of the movable tube head carries a micrometer scale extending completely around its circumference and divided into 100 equal parts, so that the length of the column of solution may be read directly to 0.01 mm. In order to prevent a change in the position of the movable head after it has been set at a certain figure, it can be fastened by means of a set screw. To minimize temperature changes the body of the tube is surrounded by an asbestos jacket. The tube is filled through a brass nipple, which is also used to insert the thermometer. The measuring scale of the tube was calibrated before use at the Bureau of Standards, and the necessary corrections, which were within 0.02 mm., were applied to all the measurements.

The special thermometer used in this work was designed to reduce to a minimum the errors caused by the exposed portion of the mercury column. The outside diameter is 5 mm., and the length over all 150 mm. The bulb is 3 cm. long. The ice point is 2 cm. above the top of the bulb. At this point the stem is graduated in tenth degrees from -1° to $+1^{\circ}$.

Shortly above the $+1^{\circ}$ point there is a mercury well. This arrangement made it possible to place the 20° mark at only 5 cm. above the top of the bulb, and here the thermometer has another graduation, in tenths, from 19° to 21° . When the thermometer was in use, the bulb was completely immersed in the liquid. Since the thermometer has a very narrow capillary, and since the measurements were made in a room kept at $20^{\circ} \pm 0.5^{\circ}$, the effect on the final result of errors in the temperature readings is very much smaller than that of the possible errors in the saccharimetric readings. The thermometer was also calibrated at the Bureau of Standards.

The sucrose used in these experiments was a part of the first lot prepared in the Bureau of Chemistry and Soils by crystallization from aqueous solution. The procedure was substantially the same as that described by Balch and Hill (p. 111), and it is therefore unnecessary to describe it here again in detail. The only important difference was that saccharimetric measurements were made on the sucrose as received, without further drying. The actual readings found, therefore, must be corrected for the moisture determined in separate portions of the sample.

It was found that moisture determinations in pure sucrose present a rather difficult problem, as has already been pointed out by Bates and Jackson¹. Drying in vacuo at around 100° , the temperature used in some experiments of Kraisy and Traegel², gave results that were obviously too high. Even at 70° , under 40 mm. absolute pressure, it was impossible to arrive at constant weight, an additional loss being observed after each hour of heating. At 50° , however, also at 40 mm. pressure, the decrease in weight stopped after two hours' heating, and in two parallel experiments the total loss in weight was found to be 0.009 and 0.010 per cent. This is evidently the maximum moisture content of the sucrose, and the actual figure may be even slightly lower.

The accuracy of ash determinations by the ordinary sulfated ash method in sucrose of highest purity is also rather problematical, because the residue obtained in the large platinum dish required for this purpose is scarcely weighable, and because the dish itself may show variations in weight of the same order as the total weight of the residue. A determination by the electrical method, however, showed a conductance corresponding to less than 0.001 per cent ash.

For the estimation of invert sugar the method of Kraisy² was used. Six determinations gave an average of 2.5 mg. of copper reduced by 10 grams of the sucrose. This would correspond to 0.002–0.003 per cent invert sugar, which is somewhat higher than the figure found by Balch and Hill with Soldaini's solution, modified. It is probable, however,

¹ Bur. Standards Scientific Paper 268, 1916.

² Z. Ver. deut. Zuckerind., 1924, 74: 193.

³ Ibid., 1921, 71: 123.

that Soldaini's method, which was previously used by Bates and Jackson, gives more reliable results. The liquid is boiled for only 2 minutes; in Kraisy's method it is boiled for 10 minutes. During this long period differences in the violence of the boiling may cause considerable variations in the quantity of copper reduced, as is also shown by comparing Kraisy's results, obtained on small quantities of added invert sugar, with those calculated from his formula.

The saccharimetric readings were made independently by two observers, C. A. Gamble and G. H. Hardin, each of whom measured ten different solutions, making twenty in all. All calculations are based on weight in vacuo of sugar and solution, and Plato's tables were used to determine the actual concentration of the solution.

The flasks were of the usual type used for sugar work; they were calibrated to a tolerance of 0.05 ml. All measurements were made on a Frič saccharimeter, Bates type, reading to 0.01° Ventzke. As the standard quartz plate used in this laboratory, according to the certificate of the Physikalisch-Technische Reichsanstalt, shows a sugar value close to that of the normal sugar solution (99.827° Ventzke, Herzfeld-Schönrock scale), it was not necessary to read the actual zero point of the saccharimeter. Only comparisons were made between the readings of the plate and those of the sugar solution.

With each of the twenty separate solutions, each observer first took five readings of the quartz plate, then five readings each for five different tube lengths between 199 and 201 mm., and finally another five readings of the quartz plate. The ten readings on the plate were averaged, and the necessary correction was applied to the average reading for each of the five tube lengths. Then the average readings were corrected to a tube length of 200.00 mm. Finally the average readings for each of the five single experiments made on the one solution were averaged and corrected to a concentration of 26 grams of sugar, weighed in air, per 100 ml. The results for each of the twenty series of tests are shown in Table 1.

The final result of the saccharimeter measurements is as follows:

Average of ten experiments by Gamble, 99.899 ± 0.004 at 19.95° .

Average of ten experiments by Hardin, 99.901 ± 0.003 at 20.01° .

The agreement between the two observers is very good, and the average result of both gives 99.900° Ventzke at 19.98° .

The temperature correction is less than 0.001° , and may therefore be disregarded. Applying a correction of 0.0095 for moisture, 0.001 for ash, and 0.0015 for reducing sugar (as found at the Bureau of Chemistry and Soils), the saccharimetric reading for the normal weight of the sucrose is found to be 99.912° Ventzke, old scale¹. This figure checks with the

¹ The average figure for Gamble and Hardin's results reported by Browne and Zerban in *This Journal*, 1928, 11 57, was arrived at by calculating the saccharimetric averages for each solution to only two decimal places and using a value of 99.83 for the quartz plate. On the other hand, only moisture was corrected for, the figure 0.01 per cent being used. This explains the discrepancies between the figures reported in the preliminary announcement and the present paper.

figure reported in Part I of this paper within 0.007° , and with that of Bates and Jackson within 0.017° . The difference appears quite reasonable when it is considered that in the final experiments of Bates and Jackson the average readings for two observers on the same instrument ranged from 99.876 to 99.930, and that in those of Balch and Hill the variation was from 99.870 to 99.923, while those obtained in this laboratory ranged from 99.868 to 99.925.

From the results of the work done in three laboratories in the United States, the writers seem justified in concluding that the true saccharimetric value of the normal weight solution of sucrose should be very close to the average value 99.904, which may be rounded off to 99.90° Ventzke, Herzfeld-Schönrock scale.

COMPARISON OF THE MONIER-WILLIAMS AND THE A. O. A. C. METHODS FOR THE DETERMINATION OF SULFUROUS ACID IN FOOD PRODUCTS.

By J. FITELSON (U. S. Food, Drug and Insecticide Administration,
Philadelphia, Pa.).

INTRODUCTION.

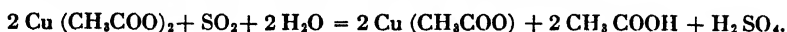
Very little work has been done by the Association of Official Agricultural Chemists on the determination of sulfurous acid, particularly in food products containing volatile sulfur compounds. Aside from the direct iodine titration method, which is applicable to but a few products, such as wine, only the direct distillation method into either iodine or bromine has been used since the earliest days of the association. However, iodine oxidizes hydrogen sulfide to sulfur and volatile organic sulfur compounds partly to sulfuric acid, and bromine oxidizes the volatile sulfur compounds to sulfuric acid to a far greater extent. Therefore, serious errors may be introduced by the use of either of these oxidizing agents. The determination of sulfurous acid contained in products such as onions, gelatine, mustard, and other spices and condiments presents special problems which have been recognized in the few attempts to modify the official A. O. A. C. method. Horne¹ suggests passing the distillate from a sulfurous acid determination through a solution of cadmium chloride, where hydrogen sulfide is precipitated as cadmium sulfide whereas sulfurous acid passes through unaffected. As the cadmium chloride wash-trap does not retain other volatile sulfur compounds, however, its use is limited.

Cupric salts have also been recommended by Winton and Bailey² as

¹ U. S. Dept. Agr. Bur. Chem. Bull. 105, p. 125.

² *J. Am. Chem. Soc.*, 1907, 29: 1499.

wash traps for sulfurous acid, but the use of these reagents is to be avoided, both because of the catalytic oxidizing effects of copper salts on sulfurous acid noted by Holleman and Cooper¹ and by Titoff² and because under appropriate conditions sulfurous acid can react chemically with copper in accordance with the following equation:



The method of Rivot³ for the determination of copper depends on the reduction of cupric salts to cuprous salts by sulfur dioxide in a solution slightly acid with hydrochloric acid and subsequent determination of the copper as cuprous sulfocyanate. The following experiments were carried out to illustrate this oxidizing effect of copper salts. A 3 per cent solution of cupric acetate, slightly acidified with hydrochloric acid, was used as the oxidizing agent in place of bromine water in the official distillation method for sulfurous acid. Known amounts of sulfurous acid, determined by direct iodine titration, were distilled over into the cupric acetate, and then determined gravimetrically as barium sulfate. A recovery of about 70 per cent sulfurous acid was obtained.

OXIDIZING AGENT	ADDED SO ₂ mg.	SO ₂ FOUND mg
Cupric acetate	13.0	9.1
	12.9	9.1

DISCUSSION OF METHOD.

Monier-Williams⁴ has shown that hydrogen peroxide in the cold oxidizes sulfurous acid to sulfuric acid quantitatively, whereas under the same conditions hydrogen sulfide and volatile organic sulfur compounds present in food products are but slightly affected. By using cold hydrogen peroxide as the oxidizing agent with the essential features of other methods for sulfurous acid determination, a method has been evolved which makes possible the accurate determination of sulfurous acid in the presence of volatile sulfur compounds. The method consists in distilling for 1 hour with a reflux condenser and sweeping the sulfurous acid into cold hydrogen peroxide by a current of carbon dioxide. Hydrochloric acid is used in place of phosphoric acid to acidify the food product, the reflux condenser preventing any hydrochloric acid from distilling over. Each of the various outstanding features of this method has its importance in an accurate determination of sulfurous acid.

In certain food products, such as dried fruits and molasses, where the sulfurous acid is in close combination with the sugars, it is difficult to effect a complete liberation of the sulfurous acid by using phosphoric

¹ Text Book of Inorganic Chemistry, 1921, p. 136.

² Z. physical Chem., 1903, 45: 641.

³ Treadwell and Hall. Analytical Chemistry, Quantitative Analysis, 1919, 2. 185.

⁴ Reports on Public Health and Medical Subjects, No. 43. Ministry of Health, London, 1927.

acid. Monier-Williams shows that in the case of dried apricots, even when relatively large quantities of hydrochloric acid are used, it takes more than 1 hour for complete distillation of the sulfurous acid. The use of hydrochloric acid insures a faster and more complete liberation of sulfurous acid from its combinations.

As a rule, volatile acids and organic sulfur compounds do not distill over when a reflux condenser is used, although when large quantities of volatile organic sulfur compounds are present, as in onions, mustard, etc., a great part of them may be carried over. The retention of the volatile organic acids in the distilling flask permits a volumetric determination of the sulfurous acid. Because of the use of a reflux, however, a longer distillation is necessary to insure the sweeping of all of the sulfurous acid into the receiver. As a routine procedure, therefore, the Monier-Williams method is somewhat lengthy, although, with only a few determinations of sulfurous acid, the method is actually rapid, due to the volumetric determination. Also, the combined effects of the use of carbon dioxide, hydrochloric acid, and a reflux condenser make frothing a minor problem in the actual manipulation. In the official A. O. A. C. method, excessive frothing retards distillation in the case of such products as glue and barley, whereas in the Monier-Williams method this frothing is quickly controlled.

Neutral hydrogen peroxide is used, thereby permitting titration of the sulfuric acid with 0.1 *N* sodium hydroxide and subsequent gravimetric determination as barium sulfate, if so desired. All manipulation of the hydrogen peroxide is carried out in the cold, since, on heating, any hydrogen sulfide or organic sulfur compound will be oxidized. Precipitation of the sulfuric acid as barium sulfate is carried out in the cold. The completeness of the oxidation of sulfurous acid, both alone and in the presence of various sulfur compounds, and the accuracy of the volumetric and gravimetric determinations of the sulfuric acid have been repeatedly verified in this laboratory.

The following advantages and disadvantages of this method may be noted. It is time-consuming, at least 1½ hours being necessary for each determination, although Monier-Williams thinks that the time of distillation can be materially reduced in the case of most food products. On the other hand, there are the following advantages: (1) The sulfurous acid is more completely liberated; (2) errors due to volatile sulfur compounds are avoided; (3) organic acids do not pass over with the distillate, thereby allowing a volumetric determination; and (4) frothing is kept to a minimum.

EXPERIMENTAL WORK.

Monier-Williams fails to show the accuracy of recovery of sulfurous acid when distilled in the presence of volatile organic sulfur compounds

from food products. He does give figures showing the extreme accuracy of this method when tested with known quantities of pure solution of sulfurous acid and with solutions of sulfurous acid containing acetaldehyde and acetic acid. He also shows the selective oxidizing power of cold hydrogen peroxide on sulfurous acid in the presence of other volatile sulfur compounds. In this laboratory the method was first checked with known quantities of sulfurous acid, a solution of sodium sulfite being used, with its sulfurous acid content determined by iodine titration. The results obtained (Table 1) agree with those of Monier-Williams and show the accuracy of the method when used under ordinary laboratory conditions.

TABLE 1.

Known quantities of sulfurous acid recovered by Monier-Williams method.

SULFUR DIOXIDE ADDED	SULFUR DIOXIDE FOUND		SULFUR DIOXIDE RECOVERY	
	By titration	Gravimetric	Gravimetric Results	
			Difference from added sulfur dioxide	Percentage recovery
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
2 20	2.35	2 00	-0 20	90 9
5.30	5 30	5.10	-0.20	96 2
10.48	11 20	11.28	+0.80	107 6
21 20	21 31	21 63	+0.43	102 0
59.18	58 94	59 88	+0 70	101 2

For the quantities of sulfurous acid with which food chemists are ordinarily concerned in food products (5 — 50 mg.), the percentage recovery is close to theoretical. The gravimetric results are considered to be more accurate, as small quantities of sulfurous acid cannot be determined accurately by titration, 1 cc. of 0.1 *N* sodium hydroxide being equivalent to 3.2 mg. of sulfurous acid.

The method was next tested with known quantities of sulfurous acid added to various food products containing volatile sulfur compounds, the sulfurous acid being added just before distillation to the flask containing the food product. Table 2 gives the results obtained.

The distillates from brown mustard and brined onions contain hydrogen sulfide and various other volatile organic sulfur compounds, which, as Monier-Williams shows, produce large quantities of sulfates when distilled into bromine or iodine, but give very small quantities of sulfates when distilled into cold hydrogen peroxide. The figures in Table 2 show that sulfurous acid is but slightly affected by volatile sulfur compounds while being distilled over. The percentage recovery of sulfurous acid is remarkably high, the maximum difference between the amount of sul-

TABLE 2.

Influence of volatile sulfur compounds on the recovery of sulfurous acid by the Monier-Williams method.

FOOD PRODUCTS	AMOUNT FOOD TAKEN	SULFUR DIOXIDE ADDED	SULFUR DIOXIDE FOUND				SULFUR DIOXIDE RECOVERY	
			By titration		Gravimetric		Corrected gravimetric results	
			Uncor- rected	Corrected for sulfurous acid in food	Uncor- rected	Corrected for sulfurous acid in food	Difference from added sulfur dioxide	Per cent recovery
No. 1 (a) Brown mustard seed	grams	mg.	mg.	mg.	mg.	mg.	mg.	
(b) " "	25	0.43	1.15
(c) " "	"	5.49	6.86	6.43	6.59	5.44	-0.05	99.1
(d) " "	"	22.97	24.95	24.62	24.92	23.77	+0.80	103.5
2 (a) Brined onions	100	0.79	0.25
(b) " "	"	2.22	2.87	2.08	2.11	1.86	-0.36	83.8
(c) " "	"	4.56	5.47	4.68	4.73	4.48	-0.08	98.3
(d) " "	"	7.84	8.99	8.20	7.96	7.71	-0.13	98.3
(e) " "	"	14.44	14.07	13.28	14.98	14.73	+0.29	102.0

furous acid recovered and the amount added being from + 0.80 to - 0.36 mg. Percentage recoveries are calculated from the sulfurous acid determined gravimetrically after correcting for the apparent sulfurous acid content of the food product. The 83.8 per cent recovery in 2 (b) is explained by the fact that only 2.22 mg. of sulfurous acid was added, whereas 0.36 mg. was lost in distillation.

A series of comparative experiments was undertaken, in order to compare the accuracy and applicability of the Monier-Williams method and the official A. O. A. C. distillation method. Monier-Williams shows the accuracy of his method with a large variety of food products. The products selected for analysis here were mainly for the purpose of showing the unreliability of the A. O. A. C. method with certain foods.

Table 3 shows that in products such as potato flour and corn sirup, where no volatile sulfur compounds are present, the determinations by the two methods are equally accurate. Products rich in sugar and containing sulfurous acid yield more sulfurous acid by the Monier-Williams method. Dried apricots (No. 5) gave about one and one-half times more sulfurous acid by the Monier-Williams method than by the A. O. A. C. method. A further distillation of these apricots for a half hour (No. 5-a) drove over a few milligrams more of the sulfurous acid, thus bearing out Monier-Williams' findings that dried fruits need more than 1 hour distillation to recover all of the sulfurous acid. A sample of glue (No. 7) yielded more sulfurous acid by the Monier-Williams

TABLE 3.

Sulfurous acid in food products as determined by Monier-Williams method and official A. O. A. C. method.

NO.	FOOD PRODUCT	AMOUNT TAKEN	SULFUR DIOXIDE FOUND		
			Monier-Williams Method		A. O. A. C. Method
			By titration	Gravimetric	
		grams	mg.	mg.	mg.
1	Molasses.....	100	nil	nil	0.27
2	Corn sirup †...	"	nil	" *	0.36
3	Dried figs.....	"	0.21	" *	" *
4	".....	"	0.23	" *	" *
5	Dried apricots ..	"	140.90	139.50	83.20
5 (a)	" " †.....	"	1.99	1.37	" *
6	Potato flour †....	20	nil	nil	nil
7	Glue.....	25	104.86	102.59	78.50
8 (a)	Brown mustard seed....	15	0.33	0.77	18.58
8 (b)	".....	25	0.43	1.15	"
9	Brined onions † ..	100	nil	0.25	"
10	" " †.....	"	nil	"	1.81
11	" " ..	"	nil	"	4.23
12	" " ..	"	0.26	0.05	0.96
13	" " ..	"	0.56	0.47	2.14
14	" " ..	"	0.76	0.19	2.06
15	" " ..	"	0.79	0.25	1.48
16	Small fresh onions ..	"	0.93	0.93	10.15
17	Imported brined onions	"	"	"	4.23
18	" " ..	"	"	"	1.81

* Blanks in columns indicate no analysis made

† Determinations made by H. Bercovitz, formerly of Philadelphia Station.

‡ Dried apricots distilled $\frac{1}{2}$ hour longer.

method than by the A. O. A. C. method. This sample was particularly high in sulfurous acid and probably required more than 1 hour distillation to drive over all of the sulfurous acid.

The greatest difference in these methods is seen in the case of products containing volatile sulfur compounds—mustard seed and onions in this case. Much of the work was done on onions, since one of the problems under investigation was to determine sulfurous acid in brined onions. Brined onions, analyzed for sulfurous acid by the official A. O. A. C. method, gave from 9.8 to 375.0 parts per million, although the presence of sulfurous acid in these onions was doubtful. Two samples of onions brined in the laboratory, known to be free from added sulfurous acid, gave 38 and 25 parts per million sulfurous acid respectively by the official method, whereas the original fresh onions gave 112 and 87 parts per million. Eight samples of imported brined onions yielded from 0.0 to 9.3 parts per million sulfurous acid by the Monier-Williams method and from 9.1 to 101.5 by the A. O. A. C. method. In order to determine conclusively whether onions develop any sulfurous acid on brining, a series of experiments was carried out. Small fresh onions were peeled and divided into three portions. One portion was placed in a 5 per cent salt

solution for 2 weeks, then transferred to a 10 per cent salt solution for another 2 weeks, and finally was brined for 2 more weeks in a 15 per cent salt solution. A second portion was given a successive brining in a 10 per cent and then a 20 per cent salt solution, and left for 4 weeks in each concentration. The third portion was brined for 8 weeks in a 20 per cent salt solution. After each interval of brining, the onions were analyzed for sulfurous acid by both methods.

TABLE 4.

Sulfurous acid in authentic brined onions.

NO.	MATERIAL	AMOUNT TAKEN	MONIER-WILLIAMS METHOD		A. O. A. C. METHOD
			By titration	Gravi- metric	
		<i>grams</i>	<i>parts per million</i>	<i>parts per million</i>	<i>parts per million</i>
1	Small fresh onions	100	9.3	9.3	101.5
2 (a)	Fresh onions (No. 1), brined for 2 weeks in 5 per cent salt solution	"	8.0	8.5	75.7
(b)	Above (No. 2a) brined 2 more weeks in 10 per cent salt solution	"	9.9	3.1	68.6
(c)	Above (No. 2b) brined 2 more weeks in 15 per cent salt solution	"	5.9	6.3	42.2
3 (a)	Fresh onions (No. 1) brined 4 weeks in 10 per cent salt solution	"	10.9	2.3	67.2
(b)	Above (No. 3a) brined 4 more weeks in 20 per cent salt solution	"	6.9	3.9	92.6
4	Fresh onions (No. 1) brined 8 weeks in 20 per cent salt solution	"	8.2	4.5	60.4
5	Brine from (No. 3b) onions	"	6.6
6	Brine from (No. 4) onions	"	25.5

Table 4 shows that little or no sulfurous acid is developed in onions during brining, so that where sulfurous acid in appreciable quantities is detected in brined onions by the Monier-Williams method, it is undoubtedly added. The sulfurous acid determined by the official A. O. A. C. method can be taken as a rough indication of the quantities of volatile sulfur compounds present in the onions, as the relative proportion of onions to brine was maintained in approximately the same relation throughout the experiments. It will thus be seen that, in general, volatile sulfur compounds are lost during brining, as would be

convenient to start with 30 per cent hydrogen peroxide, dilute somewhat, and neutralize with barium hydroxide solution, using bromphenol blue as indicator. After settling in the cold, the reagent was filtered from the barium sulfate, its strength determined by permanganate titration, and finally adjusted to a 3 per cent strength. The bromphenol blue indicator in the hydrogen peroxide remains unaffected for some time.

After connecting the apparatus, 300 cc. of distilled water, together with 20 cc. of concentrated hydrochloric acid, is introduced into the flask and boiled for a short time in a current of carbon dioxide. The food to be tested is now introduced, the procedure depending on the food. Liquids can be introduced directly by means of the dropping funnel. In the case of easily transferable solids, the flask contents are first cooled somewhat, care being taken to avoid having the hydrogen peroxide drawn up in the delivery tube (E) by regulating the flow of carbon dioxide. The food is then quickly introduced by removing the stopper (C). With semi-solid foods, requiring more time to introduce into the flask, the flask contents must be cooled by gradual immersion in cold water, and the food quickly washed in by recently boiled distilled water. After introducing the food, the mixture is boiled for 1 hour in a slow current of carbon dioxide. The flow of water in the condenser is stopped just before the end of the distillation. This causes the condenser to become hot, driving over residual traces of sulfur dioxide retained in the condenser. When the delivery tube just above the receiver (E) becomes hot to the touch, the stopper (J) is immediately removed.

The delivery tube and the Peligot tube contents are washed into the flask (F), and the liquid is titrated at room temperature with 0.1 *N* sodium hydroxide, bromphenol blue being used as indicator. The sodium hydroxide must be standardized with this indicator. Bromphenol blue is unaffected by carbon dioxide and also gives a distinct color change in cold hydrogen peroxide. One cubic centimeter of 0.1 *N* sodium hydroxide is equivalent to 3.2 mg. of sulfurous acid, so that titration of small quantities of sulfurous acid, requiring less than 0.5 cc. sodium hydroxide, is not accurate. A gravimetric determination may be made after titration, the precipitation of barium sulfate being carried out at room temperature. After settling, the supernatant liquid is filtered, and the residual barium sulfate is washed three times by decantation with boiling water. A blank should be determined on the reagents, both by titration and gravimetrically, and the results corrected accordingly.

CONCLUSIONS.

(1) The use of copper salts as a wash trap to remove sulfides from the distillate of the official A. O. A. C. sulfurous acid determination produces inaccuracies.

(2) Brining of onions reduces the proportion of volatile sulfur compounds and does not produce sulfurous acid in the onions in any appreciable quantity.

(3) The accuracy and reliability of the Monier-Williams method for determining sulfurous acid in the presence of volatile sulfur compounds has been verified.

(4) The results of a series of comparative determinations of sulfurous acid in various food products, by both the official A. O. A. C. distillation method and the Monier-Williams method, emphasize the advisability of using a method more accurate than the present official method for determining sulfurous acid in food products containing volatile sulfur compounds.

The writer wishes to acknowledge the advice given by A. M. Henry of the Philadelphia Station, Food, Drug and Insecticide Administration, in planning the work.

DETERMINATION OF HOOF MEAL¹.

By W. F. STERLING (Food Control Laboratory, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.).

INTRODUCTION.

Animal by-product feeds consist of animal tissue exclusive of hoof, horn, and hair; sometimes an appreciable quantity of vegetable tissue is present. Hoof meal is the dried and ground hoof exclusive of the inner pithy portion of the foot. Horn meal is the dried and ground horn sheath. During the process of manufacture both hoof and horn are usually mixed together previous to the drying and grinding, and the product is commonly known as hoof meal.

Those engaged in working with animal by-product feeds have been handicapped by the lack of an adequate method for the estimation of the amount of keratinous tissue contained therein. While there are several qualitative procedures that will detect the presence of hoof and horn meal when present in moderate quantity, the writer knows of no method that even approaches quantitative accuracy. The investigation reported in this paper was undertaken to develop a method that will detect the presence of hoof meal when present in an appreciable quantity, and that will give quantitative results within reasonable limits.

EXPERIMENTAL WORK.

Several methods of examination that appeared to offer some basis for solving the problem were tried. These included macroscopic and micro-

¹ Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1928.

scopic examinations, flotation, and determinations based upon differences in sulfur and cystine content. In general, it was found that these methods did not serve the purpose. In studying the properties of keratin, the principal constituent of hoof and horn, however, it was noted, according to Richter¹ and Hawk², that keratin is much less easily digested by the enzymes pepsin and trypsin than are other animal tissues.

To ascertain the possibilities of this difference of digestibility in formulating a method, a modification of the technic employed by Waterman and Johns³ in studying the rate of digestion of various proteins was tried upon the following materials:

- (1) Lean ground meat: dried, partially defatted, ground, extracted with ether, and dried.
- (2) Hoof meal: ground, extracted with ether, and dried.
- (3) Dried blood.
- (4) White connective tissue: dried and ground.
- (5) Residue from glue manufacture: largely yellow connective tissue, ground.

Each substance was ground to pass through a 40-mesh sieve. One gram samples were placed in 125 cc. Erlenmeyer flasks. Fifty cubic centimeters of 0.1 *N* hydrochloric acid containing 0.1 gram of pepsin (U. S. P.) was added, and the mixture was placed in a constant temperature oven at 37.5°C. for 24 hours; 5 cc. of 1 *N* sodium hydroxide was added and after standing 10 minutes the mixtures were filtered through a thin asbestos pad in a weighed Gooch crucible. The residues were washed with warm water and alcohol, dried to constant weight, and weighed.

There was no weighable residue from the meat, while the hoof residue weighed 0.68 gram. There was no residue from the blood and white connective tissue. The glue by-product gave a residue of 0.01 gram, which under microscopic examination was found to consist of epidermal tissue, hoof tissue, traces of cartilage, and wood fibers. When this experiment was repeated and digestion was continued for 48 hours no cartilage was found.

A sample was prepared containing 10 per cent hoof and 90 per cent meat and similarly treated. There was a residue of 0.07 gram. This residue, when compared with that from straight hoof meal, was estimated to represent a 10 per cent hoof adulteration. A sample of commercial meat meal known by factory inspection to be adulterated with 10 per cent hoof meal was treated. It gave a residue of 0.08 gram. This excessive residue is accounted for by the presence of a certain quantity of vegetable matter and the unavoidable small quantity of keratin present in many tankages.

¹ Organic Chemistry, Vol. I, 2nd ed., p. 674.

² Practical Physiological Chemistry, 2nd ed., p. 359.

³ J. Biol. Chem., 1921, 16: 9-17.

The results obtained with pepsin showed that further treatment with trypsin was unnecessary.

To test the necessity of using constant temperature during the digestion, a sample of pure meat was digested at room temperature during average midwinter weather for 48 hours. Much of the meat remained undigested. A study of the effect of longer periods of time was also made by digesting samples of equal parts of meat and hoof for 1, 2, 3, and 4 days; the residues obtained were 0.36, 0.35, 0.35, and 0.33 gram, respectively.

Because the digestion mixtures were extremely difficult to filter, several means of accelerating filtration were tried. Mixtures of meat and hoof that had been subjected to the digestion procedure were boiled; others were acidulated. In all cases much of the material in solution was reprecipitated. Digestion mixtures were placed in tubes and centrifuged. The supernatant liquor was poured off, and the residue was washed by adding warm water, shaking, and again centrifuging. After this treatment filtration was rapid. As an alternative procedure when a centrifuge is not available the supernatant liquor may be carefully decanted and the residue washed with warm water and decanted several times. This method is slower and less desirable than centrifuging.

Commercial samples of animal by-product feeds contain varying amounts of bone and occasionally slight mineral contaminations. In order to separate these materials from the meaty tissue previous to digestion a modification of the tentative method¹ for the determination of the amount of bone in tankages was used. The material is placed in a separatory funnel, and chloroform or carbon tetrachloride is added. The bone sinks to the bottom and is drawn off. The residue is washed onto a filter with the solvent, dried, and then transferred to the digestion flask. This treatment also removes most of the fats and makes further extraction unnecessary.

The following materials were treated as outlined, bone separation, centrifuging and enzymatic digestion being included in the process:

TABLE 1.
Digestion residues of authentic 1 gram samples.

	WEIGHT OF RESIDUE gram
Pure horn sheath	0.75
Pure horn sheath	0.75
Commercial meat meal	0.01
Tankage (fertilizer grade)	0.02
Hoof meal	0.68
Hoof meal	0.65
Hoof meal	0.69
Hoof meal	0.60
Hoof meal	0.64
Hoof meal	0.66

¹ *Methods of Analysis, A. O. A. C., 1925, 122, par. 30.*

In order to get a factor to calculate the amount of hoof meal in the original sample from the digestion residue the reciprocal of the average residue of the six hoof-meal samples was taken. This figure is 1.54.

METHOD.

Using the above experiments as a basis, the writer formulated the following method:

Place 1 gram of the sample, ground to pass a 40-mesh sieve, in a small separatory funnel and add sufficient chloroform or carbon tetrachloride to float the meaty portion. Draw off the sediment of bone, wash the portion that floats into a filter with the solvent used, and dry. Transfer to a 125 cc. Erlenmeyer flask. Add 50 cc. of a solution of 0.1 *N* hydrochloric acid containing 0.1 gram of pepsin, U. S. P. (This solution should be freshly prepared.) Place the mixture in a constant temperature oven or bath at 37°–40°C. for 48 hours. Add 5 cc. of 1 *N* sodium hydroxide and let stand for 10 minutes. Transfer to tubes and centrifuge. Pour off the clear liquor, wash with warm water, and centrifuge, repeating the process several times. Finally filter through a thin asbestos pad in a weighed Gooch crucible or through a weighed filter paper. Wash the residue well with warm water and finally with alcohol. Dry to constant weight and weigh. To obtain the amount of hoof meal present, multiply the weight of the residue by 1.54. Make a microscopic examination of the residue.

To test the method a series of samples was prepared containing meat, bone, tendon, and hoof meal. The quantities of hoof were unknown to the analyst previous to the determinations. The results are given in Table 2.

TABLE 2.
Results of determinations of hoof meal in test samples.

SAMPLE NO.	HOOF MEAL ADDED per cent	WEIGHT OF RESIDUE gram	HOOF MEAL AS DETERMINED per cent
1	10	0.06	9.2
2	5	0.03	4.6
3	2	0.01	1.5
4	4	0.03	4.6

Some commercial samples of animal by-product feeds contain considerable fibrous vegetable tissue, because the stomach contents are included in the raw material. This vegetable tissue appears in the residue following digestion. The microscopic examination of the residue detects this contamination, and an estimate of the amount can be made. It is hoped that a method for the quantitative separation of the hoof and vegetable tissues may be devised.

SUMMARY.

A method for the determination of hoof meal has been developed with the principle of enzymatic digestion under specific conditions as a basis. The method has been tested and found to give fairly satisfactory results when no vegetable tissue is present. A microscopic examination of the final weighed residue should be made, and when vegetable tissue is found, a correction should be applied if it is possible to estimate approximately the quantity present. Efforts are now being made to eliminate the interference of vegetable tissue.

DETERMINATION OF SMALL QUANTITIES OF NITROGEN IN PLANT MATERIALS¹.

By J. T. SULLIVAN and L. E. HORAT (State Chemist Department, Purdue University Agricultural Experiment Station, Lafayette, Ind.).

In plant materials the quantity of nitrogen present is often very low. Apple wood extracted with alcohol contains less than 0.2 per cent of nitrogen. With but a small amount of material available, accurate determinations of nitrogen by the Kjeldahl method are difficult; Phillips² points out that the determination of 2–10 mg. makes extreme care necessary. An aliquot of the alcohol extract, all of which cannot be spared, often contains less than 3.0 mg. of nitrogen.

USE OF MICROCHEMICAL METHODS.

An attempt was made to use such methods as those of Folin and Farmer³ and of Bock and Benedict⁴. These methods involve a digestion with sulfuric acid in a test tube. They are suitable for the analysis of animal products that are high in nitrogen in proportion to total organic matter; with some plant materials, on the other hand, it is necessary to take a larger sample in order to have a determinable amount of nitrogen. The large quantity of organic matter that must be digested, the danger of frothing, and the large quantity of acid required tend to make digestion in a test tube difficult. Moreover, with a liquid sample, the solvent must be evaporated before digestion, and a test tube is undesirable for this process.

DESCRIPTION OF METHODS.

Two methods were adopted, the selection by the analyst depending upon the type of sample. For perfectly dry material a method similar to that of Folin and Farmer was found to be successful. Superoxol is added to hasten the digestion in a test tube. For wet materials and extracts a small Kjeldahl flask is used. Evaporation and digestion can thus be performed in the same vessel.

Method suitable for dry material: Place the sample in a Pyrex test tube, 35 mm. × 250 mm., and add 1–2 grams of potassium sulfate, 0.02 gram of copper sulfate, and 3 cc. of concentrated sulfuric acid. Also add a few glass beads to prevent bumping and heat the mixture over a low flame. When fumes appear, cool the tube, add 5 drops of Superoxol, and continue the digestion, repeating the process of cooling and adding Superoxol until the mixture is clear. Digestion for 3 hours thereafter is sufficient. (A small funnel may be placed over the mouth of the test tube to prevent spattering.) After cooling, dilute the mixture to 30 cc. with ammonia-free water, cool, and add a few drops of caprylic alcohol. Neutralize the mixture with 10 cc. of saturated (75 per

¹ Published with the approval of the Director of the Agricultural Experiment Station.

² *Plant Physiology*, 1927, 2: 205.

³ *J. Biol. Chem.*, 1912, 11: 493.

⁴ *Ibid.*, 1915, 23: 47.

cent) sodium hydroxide and aerate into 0.02 *N* sulfuric acid in the Van Slyke-Cullen urea apparatus¹. (The strong alkali runs to the bottom of the tube and does not mix with the acid until the stopper is closed and air is drawn through.) Allow the aeration to proceed slowly while the mixture is hot, but after cooling speed it up as rapidly as is possible without danger of spattering. A 2 hour period of aeration is sufficient. Make a back titration with 0.02 *N* sodium hydroxide, using methyl red as indicator. A blank is usually less than a drop of standard acid.

Plant extracts, especially those of woody plants, contain substances that froth during digestion with sulfuric acid. The presence of water also causes frothing. With extracts it is desirable to evaporate the solvent in the same vessel in which digestion is to take place. For these reasons a 250 cc. Kjeldahl flask, instead of a test tube, is preferred.

Method suitable for extracts: Boil off the water or alcohol from the acidified extract and continue digestion as performed in the previous method except to use a 250 cc. Kjeldahl flask instead of a test tube. Constant attention may be avoided if the Superoxol is omitted and more sulfuric acid is added. If 5 cc. of acid is used, enough surface of the bottom of the flask will be covered to allow heating with a stronger flame and complete the digestion 1 hour after the mixture is clear. (Many samples require additional quantities of acid.) After cooling, transfer the mixture to a test tube and wash the flask with successive portions of water until a volume of 30-40 cc. is reached. After cooling, add 5 cc. of saturated sodium hydroxide and cool the mixture again. Effect final neutralization with 10 cc. of alkali, and perform the aeration in the Van Slyke-Cullen urea apparatus. Use 15 cc. of alkali to neutralize 5 cc. of sulfuric acid, but make the addition in two fractions to prevent too high a temperature during aeration.

Results of determinations by these two methods, compared with those obtained by the official Kjeldahl method², are shown in the table.

TABLE 1.
Results of nitrogen determinations.

DRY APPLE WOOD		APPLE WOOD EXTRACT	
KJELDAHL METHOD ON 1.0 GRAM	MICRO METHOD ON 0.1 GRAM	KJELDAHL METHOD ON 500 CC.	MICRO METHOD ON 50 CC.
mg.	mg.	mg.	mg.
10.76	1.049	7.79	0.779
10.69	1.060	7.76	0.804
10.93	1.068	7.86	0.780
10.71	1.079	7.90	0.789
10.83	1.068	7.87	0.807
10.72	1.046	7.76	0.792
10.60	1.068	7.76	0.801
10.40	1.090	7.73	0.810
10.84	1.079	8.01	0.801
10.75	1.072		0.795
10.73	1.078		0.789
	1.050		
	1.068		
	1.036		
	1.068		
Average 10.72	1.065	7.83	0.795

¹ *J. Biol. Chem.*, 1914, 19: 211.

² *Methods of Analysis*, A. O. A. C., 1925, 6-8.

SUMMARY.

Plant materials, high in organic matter but low in nitrogen, may be analyzed for nitrogen by the methods suggested in this paper with as high an accuracy as the regular Kjeldahl method will give with larger samples. Dry material may be digested in a test tube with the aid of Superoxol. Extracts may be evaporated to dryness and digested in a small Kjeldahl flask and then transferred to a test tube for the aeration of the ammonia.

BIOASSAY OF COMMERCIAL PITUITARY POWDERS¹.

By W. T. McCLOSKY and J. C. MUNCH² (Pharmacological Laboratory, Food, Drug and Insecticide Administration, Washington, D. C.).

Glandular products are finding their way into therapeutics and some of them have found their way into the U. S. Pharmacopeia. One of these products is the cleaned, dried and powdered posterior pituitary lobes of domesticated animals used for food by man and is known in U. S. P. X. as "pituitarium". No mention is made in the Pharmacopeia as to the physiological strength of pituitarium.

Accordingly a number of commercial samples of pituitarium, representing the current output, were obtained directly from the manufacturers, and a study of their activities was made. In addition, samples of anterior pituitary and whole pituitary powders were obtained from the same sources and studied for oxytocic activity.

Commercial whole pituitary powders showed a ratio of activity of one part of posterior substance in eight parts of whole body when assayed by the U. S. P. X method on the isolated guinea pig uterus. The standard posterior powder was used as a basis of comparison. The extracts, which were made according to the U. S. P. directions for liquor pituitarii, contained the activity of 10 mg. of powder in 1 cc. of solution.

Commercial anterior pituitary powders were uniformly inactive when assayed by the U. S. P. X method on the guinea pig uterus. Several tests of the extracts, made as above stated, showed no fall of blood pressure in an anesthetized dog in doses of 10 mg. per kilo.

Commercial posterior pituitary powders showed varying activities. Extracts were made and assayed by the method specified in U. S. P. X for liquor pituitarii. The results are given in the table.

The figures reported shows that the activity of the majority of the powders assayed is between 30 and 50 per cent of the standard posterior pituitary powder, U. S. P. X; therefore the suggestion is made that 50 per cent be established as the standard of activity of pituitarium.

¹ Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1928.

² Resigned January 1, 1928.

Assay of commercial posterior pituitary powders.

P. C. NO.	ORIGINAL CONCENTRATION PREPARED	CONCENTRATION FOUND BY U. S. P. X BIOASSAY	COMMERCIAL POWDER EQUIVALENT TO 1 MG. U. S. P. X STANDARD POWDER	ACTIVITY- STANDARD POWDER
	mg./cc.	mg./cc.	mg.	per cent
1489	10.0	0.15	66.7	1.5
1544	1.0	0.25	4.0	25.0
1483	10.0	3.00	3.3	30.0
1548	10.0	3.60	2.80	36.0
1607	10.0	3.60	2.80	36.0
1556	10.0	5.00	2.0	50.0
1517	10.0	6.00	1.67	60.0
1463	10.0	7.50	1.33	75.0

CONCLUSIONS.

1. It is suggested that the method of the U. S. P. X for the assay of liquor pituitarii be made a tentative method of the A. O. A. C. for the assay of pituitarium, U. S. P. X, as well as for the assay of desiccated whole pituitary powder.

2. The following standards for physiological activity are suggested:

Pituitarium, U. S. P.—50 per cent of the activity of the U. S. P. X official standard posterior pituitary powder.

Desiccated whole pituitary powder—5 per cent of the activity of the U. S. P. X official standard posterior pituitary powder.

BOOK REVIEWS.

Fertilizers. The source, character and composition of fertilizer materials, and suggestions as to their use. By the late EDWARD B. VOORHEES. Second revised edition by Sidney B. Haskell. The Macmillan Company, New York, 1926. Price \$2.50.

Mr. Haskell has done a real service in revising and bringing up to date Voorhees' most excellent book on fertilizers and "in so doing to leave the book true to its original concept", as he so aptly phrases it in his preface to this edition.

First published in 1898, the book was reprinted with corrections in 1900. Such was its popularity and the demand for it that it was reprinted practically every year from 1902 to 1914. The author then revised the book, and the revision was printed in 1916 and reprinted in 1917.

Since the 1916 revision there has been great growth and development in the fertilizer industry, in the use of more concentrated fertilizers, and in field experiments with fertilizers. These factors, together with the popularity of the book, made a later revision highly desirable, and, indeed, necessary, in order to bring it abreast of the times.

The scope of the book is indicated by its full title, and shown by the chapter headings, which Mr. Haskell has expanded from 15 to 17: I—The Natural Fertility of the Soil, and Sources of Loss of the Elements of Fertility; II—The Function of Manures and Fertilizers, and the Need of Artificial Fertilizers; III—Nitrogenous Fertilizers; IV—Phosphates—Their Sources, Composition, and Relative Value; V—Superphosphates—Potash; VI—Miscellaneous Fertilizing Materials; VII—Farmyard and Green Manures; VIII—Lime and Calcium Compounds; IX—Purchase of Fertilizers; X—The Valuation of Fertilizers; XI—Concentrated Fertilizers; XII—Methods of Use of Fertilizers; XIII—Fertilizers for Cereals and Grasses; XIV—Field Truck Crops; XV—Market-Garden Crops; XVI—Tree-Fruits and Berries; XVII—Fertilizers for Various Special Crops.

Chapter XI is, of course, new; Chapters VII and VIII are an expansion of Chapter VI in the 1916 edition. These changes, together with a rearrangement of the remaining chapters, have resulted in a net increase of two chapters over the 1916 edition.

The value of the book has been enhanced by the introduction of a number of well-chosen illustrations.

Written primarily for the general reader and practical man, Voorhees' Fertilizers is free from unnecessary technical terms and is eminently practical. It should be in the hands of every purchaser and user of fertilizers, who will find its contents enlightening both in regard to the nature of soil fertility and to the sources and nature of fertilizing materials, and a safe and sane guide to the intelligent purchase and use of commercial fertilizers.

This book would be a valuable text in high schools, especially where agricultural courses are offered, and certainly in agricultural schools.—R. N. BRACKETT.

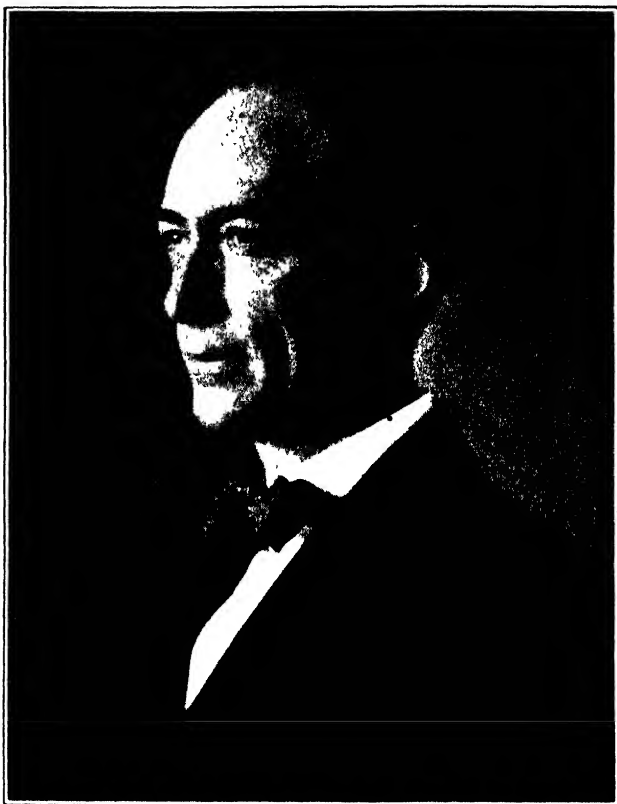
NEW BOOKS.

Wheat Flour and Diet. By C. O. Swanson. XIII + 203 pages. The Macmillan Company, New York, 1928. Price \$2.50. A popular discussion of human nutrition and a description of milling processes. It should prove useful to students and teachers of home economics, diet and nutrition, and milling processes.

The Farm. By Eugene Davenport. XIX + 462 pages. The Macmillan Company, New York, 1927. Price \$3.50. One of the Rural Science Series, edited by L. H. Bailey.

A book that evaluates the changing situation of the farmer produced by the bewildering wealth of discovery and invention at his disposal. Not a handbook of detailed agricultural practices but a "discussion of the principles controlling agricultural operations".

The Foundations of Nutrition. By Mary Swartz Rose. XI + 501 pages. The Macmillan Company, New York, 1928. Price \$2.75. Since an effort has been made to present, within a small space, some of the fundamental principles of human nutrition in simple terms, this book will prove of value to the layman who wishes to live more intelligently.



JOHN KERFOOT HAYWOOD, 1874-1928

JOHN KERFOOT HAYWOOD

On the morning of January 13, 1897, there appeared at the old remodeled dwelling on the corner of 14th and B Streets which housed the Division of Chemistry, a slender, yellow-haired, blue-eyed youth, who announced to the young lady chief clerk that he was John Kerfoot Haywood, the assistant chemist recently appointed to Doctor Wiley's staff. He was taken in to see the "Old Man", and owing to his engaging personality, ready wit and contagious good humor, promptly registered a favorite with the Chief and took his place as the youngest member of that coterie of congenial spirits in the old chemistry unit known as "Wiley's Boys". Such was the entrance into the Government service of "Jake", so dubbed by his friends from the initials of his name—a generous, lovable, warm-hearted friend and comrade.

A scion of the distinguished Haywood Family of the Carolinas, John Kerfoot was born at Raleigh, December 19, 1874, to Margaret Henry and Edward Graham Haywood. He lost both of his parents while still a youth and came to live with relatives in Washington, where he received his early education in the public schools. He entered Cornell University in 1892, and was graduated with honors in 1896. During his senior year he acted as assistant chemist at the Cornell Experiment Station, and upon his graduation was appointed instructor in chemistry. With the exception, then, of the six months he served as instructor at Cornell University, his entire professional life was spent in the service of the U. S. Department of Agriculture. Although he led an active life in his early Departmental work and progressed rapidly, his ambition to prepare himself for bigger and better things led him to take a medical course at George Washington University, from which institution he graduated, receiving the degree of Doctor of Medicine, in 1907. He was awarded the Faculty Prize for the highest average standing throughout the four-year course, his mark being the highest ever attained by a student in medicine at that institution.

At the time Haywood joined the Division of Chemistry an unusual opportunity for training was afforded young chemists by association with Doctor Wiley and such well-known scientists as E. E. Ewell, Gilbert L. Spencer, W. D. Bigelow, and K. C. McElroy. In those days the work was not organized on a subject matter basis; each man had a special assignment. Haywood's first detail was with the well-known sugar expert, G. L. Spencer, and for a youngster just out of college it was a tough assignment. Spencer was a kindly man, but he was a hard task-master, and as Haywood afterward expressed it, the first six months he "sweat blood" in the effort to make good—but make good he did, and in doing so gave evidence of that brilliant mind, thorough training, and unusual laboratory technic that did credit to Dennis, Orndorff, and Bancroft, his preceptors at Cornell.

It happened that I was also working on a temporary assignment with Spencer when Haywood arrived, and at once there arose a mutual understanding that developed into a friendship, and finally into that peculiar camaraderie that begets a feeling of "mine is thine". Fortunate indeed

is the man that has experienced such a friendship. It was from that point of contact that I was able to follow and appreciate the brilliant career of John K. Haywood.

Having attracted the attention of his Chief by the splendid record he had made with Spencer, Haywood was given other and more important work, where his initiative, industry, and keen perception made it apparent that he was of the pioneer type, a true investigator. Then he received his first big assignment—the problem of the damage caused by smelter fumes in the National Forests, which was referred to the Division of Chemistry for investigation. It was almost a virgin field in this country, although considerable work had been done in Germany and in Russia. Haywood culled the world's literature for information and inspiration. Many a weary evening hour was spent in digging into such intricate German as that of Haselhoff and Lindau, but he mastered the subject and organized the work which resulted in the investigation of smelter damage at Anaconda, Montana; Redding, California; and Ducktown, Tennessee. As a result of this work the Government was able to secure court decisions to abate this nuisance, thereby protecting the public forests. In addition two bulletins, Bureau of Chemistry Bulletins 89 and 113, which are pioneer publications in this field and are still regarded as authoritative, were issued. Out of this investigation grew an amusing instance, which Haywood always liked to relate. He, with others, was called by President Roosevelt to the White House for a conference on the smelter damage. The conference was attended by Senators and other important personages and lasted for some time. Haywood was finally called upon. Noting the President's growing impatience, he gave the results of his investigations in a brief and business-like manner, in striking contrast to the verbosity of his distinguished predecessors. When leaving he was surprised to be greeted by the President with an unusually vigorous handshake, a flash of the famous Roosevelt smile, and "I like you, young man—you say what you have to say and shut up".

In the reorganization of the Department of Agriculture in 1901, the Division of Chemistry was made the Bureau of Chemistry, with three major divisions, and laboratories reporting directly to the Chief. The Division of Foods was created with W. D. Bigelow in charge; the Division of Drugs, with L. F. Kebler in charge; and the Miscellaneous Division, with J. K. Haywood in charge. To the Miscellaneous Division was assigned the research work on insecticides and fungicides, which Haywood had organized, on smelter fumes, on cattle foods, and on irrigating waters. Here again Haywood saw an opportunity to do pioneering work in the field of insecticide and fungicide chemistry. In fact, it may be said that he and his colleagues working in the Association of Official Agricultural Chemists created an insecticide chemistry. Among his many publications in this field, perhaps the most noteworthy are Bureau of Chemistry Bulletin 68 and *Farmers' Bulletin* 146.

The work of Haywood and his colleagues, showing the sophistication of insecticides and fungicides, and his efforts in bringing the matter prominently to the attention of horticulturists and entomologists created a demand for remedial Federal legislation on the subject that culminated in the passing of the Federal Insecticide Act of 1910. The enforcement of the act was assigned to the Department of Agriculture, a Board in the Department being created for this purpose. Secretary James Wilson appointed Haywood to represent the Bureau of Chemistry on this Board,

and as the work was largely chemical, he exercised a determining influence in organizing the inspection work and forming the policies for the enforcement of this law. Later he was made Chairman of the Board. As he was an administrator of a forceful, energetic, somewhat domineering type, the kind that hews to the line, let the chips fall where they may, his activities in securing a compliance with the provisions of the new act met with some vigorous opposition among a few unethical manufacturers, and this opposition was reflected both within and without the Department. On several occasions strong pressure was brought to remove him from office. He never faltered, however, but fought on with increased energy under an inspiring banner, inscribed "Conquer we must for our cause it is just", and he had the great satisfaction in his later years of having some of his most vigorous early opponents publicly acknowledge that in his fight for a higher ethical standard among manufacturers of insecticides and fungicides he had rendered a great and lasting service to his opponents, to the industry, and to the public.

During the early and constructive period of his career, Haywood was the author of some forty scientific and technical papers. Later in life his whole time was given to the administration of the insecticide law. He was the recipient of numerous honors. He was president of the Association of Official Agricultural Chemists in 1917, after having served the association in many offices including the chairmanship of the first Committee on Revision of Methods. He was a past president of the Feed Control Officials; Vice-President of the Medical Control Officials; Chairman of the Jury of Awards, St. Louis Exposition; and a member of Sigma Xi. He was a Mason and a member of the Episcopal Church.

On November 3, 1928, at Emergency Hospital in Washington, D. C., Haywood succumbed to an acute attack of bronchitis. He was laid to rest in old Rock Creek Cemetery, while gathered around his bier a loving, loyal band of comrades joined feelingly with friends and relatives in the response, "Father, in thy gracious keeping, leave we now thy servant sleeping".

W. W. SKINNER.

FIRST DAY. MONDAY—MORNING SESSION.

No report on waters, brine, and salt was given by the referee.

No report on tanning materials and leathers was given by the referee.

REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

The work of the Referee on Insecticides and Fungicides for 1928 consisted of a further study of the tentative electrolytic method for the determination of copper¹ in such products as Bordeaux-Paris green and Bordeaux-calcium arsenate.

This method has been adopted by the association as official (first action)². It provides for the deposition of the copper from nitric acid solution without the previous removal of arsenic. The latter is oxidized to the pentavalent condition and kept in this state during the electrolysis by hydrogen peroxide. This action prevents its deposition with the copper.

It was found that when 3 per cent hydrogen peroxide was used in the quantities directed the deposition of the copper was slow until the concentration of the peroxide had been reduced by the electrolysis. Less peroxide was added, and no further trouble was experienced. With this slight change, the method reads as follows:

Dissolve 2 grams of the powdered sample contained in a 150 cc beaker with 5 cc. of strong nitric acid, add 25 cc. of a 3 per cent solution of hydrogen peroxide, and warm on a steam bath for 5–10 minutes. Add 25 cc. of hydrogen peroxide solution; dilute to about 100 cc.; and electrolyze, using a weighed gauze cathode, a rotating paddle anode, and a current of 2–3 amperes. At the end of about 20 minutes, add 15–20 cc. more of the hydrogen peroxide solution. After all copper is deposited, which should not require more than 45 minutes, and while the current is still flowing, wash the deposit with water by siphoning. Then interrupt the current, rinse with alcohol, dry for a few minutes in an oven, weigh, and calculate the percentage of copper. (Do not pass the current for more than 5–10 minutes after all copper is deposited without adding more of the hydrogen peroxide solution.)

A sample of Bordeaux-Paris green was prepared, and copper was determined by three chemists by the method as outlined. In order to have comparative results, the referee and one other analyst also determined the copper by thiosulfate titration.

¹ *Methods of Analysis*, A. O. A. C., 1925, 63, par. 87.

² *This Journal*, 1924, 7-261.

The collaborative results are given in Table 1.

TABLE 1.
Collaborative results on Bordeaux-Paris green mixture.

ANALYST	COPPER	
	TENTATIVE METHOD	THIOSULFATE METHOD
	<i>per cent</i>	<i>per cent</i>
J. C. Bubb, Food, Drug and Insecticide Administration, Washington, D. C.	12.49	12.43
	12.49	12.53
	12.49
	12.45
Average	12.48	12.48
J. J. T. Graham	12.55	12.46
	12.47	12.46
	12.46
	12.51
Average	12.50	12.46
A. C. Nothstine, Food, Drug and Insecticide Administration, Washington, D. C.	12.44
General Average	12.49	12.47

DISCUSSION OF RESULTS.

The difference between the general averages obtained by the use of the modified tentative method and the thiosulfate titration method is only 0.02 per cent. The maximum variation in the results obtained with the modified tentative method by the three analysts was 0.11 per cent. These results are very satisfactory, and they check with those obtained by the thiosulfate method. The work this year indicates that the modified tentative method is reliable and should be made official.

RECOMMENDATION FOR FUTURE WORK.

Chlorinated lime, coal-tar dips, non-soap oil emulsions, miscible oils, and organic-mercury seed disinfectants are important insecticides and fungicides, and it is recommended that methods for their analysis receive early consideration.

RECOMMENDATIONS¹.

It is recommended—

(1) That the tentative method for copper in Bordeaux-Paris green and Bordeaux-calcium arsenate, as revised in this report, be adopted as official.

(2) That the tentative method for the determination of unsulfonated residue in mineral oils and in the recovered oil obtained in the analysis of oil emulsions, as amended at the 1927 meeting², be adopted as official (final action).

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 65.

² *This Journal*, 1928, 11: 146.

REPORT ON FLUORINE COMPOUNDS.

By G. A. SHUEY¹ (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*.

In his previous report² the associate referee briefly discussed four methods for the determination of fluorine, to which he had given some attention. Of these methods the so-called "volatilization" method proved to be superior to the others in point of speed, also in applicability to the analysis of numerous fluorine-bearing compounds. On the basis of the preliminary studies that were made in the laboratory of the associate referee, therefore, work was confined to a further study of the volatilization method.

Considerable time was devoted to the study of temperature, time of heating, agitation, strength and quantity of acid, and form and amount of silica required by different fluorine compounds in order to prescribe conditions that will improve the accuracy of the method.

The associate referee has designed a flask which is considered an improvement because it permits the introduction of sulfuric acid without removal of the stopper and thus prevents contamination by atmospheric moisture. The flask may be heated directly over the burner flame.

Delay was experienced in procuring and standardizing the flask. Five collaborators are now actively engaged in a study of the volatilization method, but their results have not been received. Results obtained by the associate referee are very encouraging. The work is to be continued, and all data, with recommendations, will be submitted to the association at its next meeting.

REPORT ON CAUSTIC POISONS.

By C. M. SMITH (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

Many states have passed laws regulating the sale of dangerous caustic or corrosive substances, and on March 4, 1927, the Federal Caustic Poison Act went into effect. At the 1927 meeting of this association it was decided to undertake the study of methods of analysis for such materials, and the writer was appointed general referee.

In the enforcement of the Federal Caustic Poison Act during the past year most of the analytical work dealt with the determination of benzo-phenol in such products as saponified cresol solutions, coal-tar dips, disinfectants, fly sprays, etc. It was found that cresol, even when distilled at the high temperatures specified by the U. S. P., may contain large

¹ Presented by J. J. T. Graham.

² *This Journal*, 1928, 11: 147

percentages of phenol, and hence every product containing tar acids must be examined for the latter. The testing of methods for the determination is the subject of this report.

A survey of the literature indicated that for the determination of moderate proportions of benzophenol in the presence of cresols and other higher homologs, the procedure outlined by R. M. Chapin¹ appeared to offer the greatest promise.

This method consists essentially in treating the phenol solution with Millon's reagent, thereby developing a reddish color, the intensity of which is then compared with standards made from C. P. phenol.

The collaborative work undertaken as a preliminary test of the suitability of this method for the purposes of the association was confined to members of this laboratory.

The method was first tested by E. L. Griffin by analyzing a commercial cresol for its phenol content, and then preparing and analyzing two mixtures of this product and C. P. phenol.

The results obtained are shown in Table 1.

TABLE 1.
Results of analysis obtained by Griffin.

MATERIAL TESTED	PHENOL	
	THEORETICAL per cent	FOUND per cent
Sample of cresol.....	. .	3.5
95% cresol + 5% phenol.....	8 4	8 0
75% cresol + 25% phenol.....	27 6	26 5

Next, an emulsifiable product was made from soda-rosin soap and a coal tar neutral oil from which the tar acids had been carefully extracted. The product itself showed 0.3 per cent phenol, and when mixed with 5 per cent C. P. phenol the mixture gave 4.9 per cent by Chapin's method. Finally, a kerosene mixture known to contain 11.0 per cent of phenol furnished 10.0 per cent by Chapin's method. These experiments indicate that the method is correct to about 1 part in 10, which is fairly satisfactory for a colorimetric procedure. Further, they show that no interference is caused by kerosene, neutral oils, or soda-rosin soap.

Four products were prepared from known quantities of soap, cresol, neutral oil, and phenol. The constituents used had the following characteristics:

Soap.—Neutral potash linseed oil soap containing 20% water.

Neutral Oil.—A 25 per cent creosote oil was freed from phenol by 8 extractions with 10 per cent sodium hydroxide solution and was then washed with salt solution, dried on the steam bath, and filtered.

¹ U. S. Dept. Agr. Bull. 1308.

Cresol.—A commercial cresol was diluted with ether and extracted 3 times with 10 per cent sodium hydroxide solution, such quantity of the alkali being used that in all about $\frac{1}{2}$ of the tar acids was extracted. The remaining ether solution was washed twice with water and separated, the ether was evaporated, and the remaining cresol was fractionated. The third quarter of the distillate was kept for use.

Phenol.—C. P. material with melting point of 40.4°C.

Analyses were made by E. H. Hamilton and by the referee. Entirely different reagents, including different samples of crystallized phenol, were used in making the standards. In all cases, duplicate analyses showed satisfactory agreement. The results obtained are given in Table 2.

TABLE 2.

Results of analyses obtained by Hamilton and Smith.

MIXTURE	SOAP <i>grams</i>	CRESOL <i>grams</i>	NEUTRAL OIL <i>grams</i>	PHENOL— THEORETICAL <i>per cent</i>	PHENOL—DETERMINED	
					E. H. HAMILTON <i>per cent</i>	C. M. SMITH <i>per cent</i>
1	25	12	63	0.0	0.2	0.3
2	25	12	58	5.0	4.8	4.7
3	25	12	56	7.0	6.4	6.6
4	25	12	53	10.0	9.1	9.0

The agreement in the results is quite satisfactory, but the tendency to low values is still evident.

Since these tests were made the method has been used on about 200 samples, without, however, any check against other methods.

Chapin states that betanaphthol and salicylic acid invalidate this test. The writer has encountered one case of a fly spray perfumed with methyl salicylate and has confirmed his statement so far as the salicylate is concerned. Two methods for overcoming this interference have been studied: one consists in saponifying the methyl salicylate with sodium bicarbonate and removing the salicylic acid as sodium salicylate, and the other depends on extraction of the methyl salicylate itself with kerosene. Both of these methods gave promising results and will be studied in greater detail later.

DISCUSSION.

The experiments described in this report indicate that Chapin's method for the estimation of benzophenol is satisfactory for use with disinfectants and other similar phenolic solutions coming within the purview of the Federal and State caustic poison acts. Such mixtures may generally be analyzed directly without the preliminary separation of the total phenols. The precision of the results is only about one part in ten, but the referee considers that this is satisfactory when it is remembered that this is an attempt to estimate a material in the presence

of several of its homologs. A tendency to give slightly low results was recognized by Chapin and has been confirmed by the referee.

SUGGESTIONS FOR FUTURE WORK.

Since the Federal Caustic Poison Act specifically includes several acids and alkalies when "free or chemically unneutralized" and ammonia when "free or chemically uncombined", it is desirable that this association consider methods for the determination of all such constituents. Ordinary solutions of acids and alkalies will of course offer no difficulty, but the presence of salts of heavy metals, as for example of zinc in soldering fluids and of copper in ammoniacal copper fungicides, requires special consideration.

RECOMMENDATIONS¹.

It is recommended—

(1) That the Chapin method for the estimation of benzophenol be subjected to further collaborative study.

(2) That the effect of salicylates on the Chapin method be studied, and that procedures for overcoming it be tested by collaborative work.

REPORT ON SOILS AND LIMING MATERIALS.

By W. H. MACINTIRE (Agricultural Experiment Station, Knoxville, Tenn.), *Referee*.

RECOMMENDATIONS¹.

It is recommended—

(1) That the modified sugar method² for the determination of the caustic value of lime be adopted as official (final action).

(2) That section 18³ be captioned "Oxides of iron, aluminum, manganese, phosphorus and titanium" instead of "Ferric and aluminium and titanium oxides and phosphorus", and that co-precipitation of manganese⁴ be provided for by the following insertions and changes:

(a) After the first sentence insert the sentence, "Add 0.5 gram of solid ammonium persulfate":

(b) Change the next to the last sentence to read: "Reprecipitate the oxides with ammonium persulfate and dilute ammonium hydroxide as directed above, etc".

(c) In the second sentence of the second paragraph insert "manganomanganic oxide (Mn_3O_4)" between (Al_2O_3) and titanium oxide.

(d) Preceding the last paragraph insert a paragraph to read as follows: "Evaporate 50–100 cc. of the solution from 17 after the addition of 10 cc. of concentrated nitric

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 66.

² *This Journal*, 1928, 11: 153; *Ind. Eng. Chem.*, 1928, 20: 312, 315.

³ *Methods of Analysis*, A. O. A. C., 1925, 28.

⁴ *The Chemical Analysis of Rocks*, 3rd ed. Wiley and Sons.

acid. Repeat the addition of nitric acid and evaporation to insure expulsion of all hydrochloric acid. From this point proceed as directed in 75, p. 101.

(e) Change the last paragraph to read as follows: "Subtract the sum of the oxides of iron, manganese, and phosphorus (determined separately as directed under 27 or 28) from the weight of the combined oxides of iron, aluminum, manganese, phosphorus, and titanium determined as directed above. Report the remainder as oxides of aluminum and titanium".

(3) That the periodate method for the determination of manganese, when this element only is to be determined, be further studied in comparison with the present tentative method.

(4) That the question of hydrogen-ion concentration in soil systems be given recognition in the next edition of *Methods of Analysis* and that the alternative specific acidity and specific alkalinity method of expression be used in addition to the Sorensen values.

No report on the reaction value of alkaline soil was given by the associate referee.

REPORT ON REACTION VALUE OF ACID SOILS.

By E. T. WHERRY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

No analytical work is to be reported here, but at the request of the Referee on Soils and Liming Materials a recommendation is made in regard to the method of stating the results of soil reaction measurement.

Soil reaction data are at the present day commonly stated in pH numbers, which are the reciprocal logarithms of the concentration of hydrogen ion. Accordingly, the smaller the number the greater the acidity, and each pH unit corresponds to ten times as much hydrogen ion as the one next below. This situation makes it exceedingly difficult for laymen to appreciate the magnitude of the active acidity in a given case, and indeed instances are known where technical men themselves have drawn unsound conclusions from using pH numbers as if they were arithmetically instead of logarithmically related. The following tentative plan is accordingly recommended¹:

SOIL REACTION.

Method of statement.—In stating reaction values of soils, the pH numbers should be supplemented by the corresponding values of hydrogen- or hydroxyl-ion concentration. The most convenient way to express these is to make use of units of 10^{-7} grams per liter of hydrogen ion,

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12 63, 66.

and 17×10^{-7} grams per liter of hydroxyl ion. These units represent the ionic concentrations in pure water at about 18° , a temperature selected to make the exponent a whole number.

To calculate hydrogen-ion concentrations in these units, the following formulas may be used:

$$H^+ = 10^{(7-pH)} - 1/10^{(7-pH)}; OH^- = 10^{(pH-7)} - 1/10^{(pH-7)}.$$

In both cases, when the first of the two terms is greater than 10, the second term may be neglected. A table showing rounded off equivalents of pH values often met with has been published¹.

REPORT ON LIMING MATERIALS.

By W. M. SHAW² (Agricultural Experiment Station, Knoxville, Tenn.),
Associate Referee.

The associate referee has devoted considerable time to the fundamental studies of the action of calcium hydroxide solutions upon colloidal silica and silica gel. Some new and interesting observations have been made and are now being shaped for publication.

It is recommended³ that the modified sugar method for the determination of available lime be adopted as official (final action).

REPORT ON LESS COMMON METALS IN SOILS.

By J. S. MCHARGUE (Agricultural Experiment Station, Lexington, Ky.),
Associate Referee.

Eight different Experiment Stations agreed to do cooperative work upon proposed methods for the estimation of manganese, copper, zinc, nickel, and cobalt in soils and plants. Accordingly, three samples of soil and six samples of plant material were sent out, together with copies of the proposed methods.

Two laboratories only made reports. One laboratory made manganese determinations on all the samples sent. The other laboratory made manganese determinations on the soils, the results being as follows:

	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Agricultural Experiment Station, Purdue, Ind. . . .	0 1515	0.1875	0 2269
Agricultural Experiment Station, Kingston, R. I. . .	0 1845	0 2203	0.2524
Agricultural Experiment Station, Lexington, Ky.: .			
Shedd	0 2360	0 2170	0.2840
Roy	0 2000	0 2500	0 2500
Theoretical	0 2000	0 2500	0 3000

¹ *This Journal*, 1929, 12: 64.

² Presented by W. H. MacIntire.

³ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 66.

It is recommended¹ that the following bisulfate fusion method for the determination of manganese in soils be incorporated in the methods for soil analysis.

MANGANESE IN SOILS.

REAGENTS.

- (a) *Sulfuric acid solution* (1 + 1).
- (b) *Potassium bisulfate*.—Manganese-free and finely pulverized.
- (c) *Standard manganous sulfate solution*.—Dissolve 0.1877 gram of pure potassium permanganate in about 100 cc. of water, acidify the solution with dilute sulfuric acid (1 + 1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10 per cent oxalic acid solution to discharge the color. Cool and dilute to 1 liter. 1 cc. of this solution = 0.1 mg. of manganese.
- (d) *Potassium periodate*.

DETERMINATION.

Weigh 0.5–5.0 grams of finely pulverized, air-dried soil into a 50 cc. silica or platinum crucible. Add to the soil approximately $2\frac{1}{2}$ times its weight of finely powdered, manganese-free potassium bisulfate and mix thoroughly. Place the lid on the crucible and heat gently over a Bunsen burner for about 5 minutes; increase the heat gradually until the crucible and lid are red hot, being careful not to allow the contents of the crucible to froth over. Continue to heat for about 20 minutes, or until the frothing has ceased and the contents are in a quiet molten condition. Withdraw the flame from beneath the crucible, remove the lid, and rotate the crucible in a horizontal position to spread the molten contents over the inner walls to expedite cooling. When the crucible is no longer red, immerse it in about 25 cc. of sulfuric acid (1 + 1) in a 250 cc. beaker and digest over a hole on a hot water bath until the contents of the crucible disintegrate and dissolve. Carefully rinse the crucible and lid with hot water and dilute the solution to about 100 cc. Filter, and wash the insoluble residue.

Discard the insoluble residue if it has a uniform white color. If it is colored by undecomposed particles of minerals, ignite and expel the silica with hydrofluoric and sulfuric acids. Fuse the residue with potassium bisulfate, digest in dilute sulfuric acid, and add the solution to the first filtrate from the fusion.

Make the solution that contains all the manganese to a definite volume and take an aliquot for the determination. Add about 0.05 gram of potassium periodate to the aliquot. Boil the solution until the characteristic purplish permanganic acid color develops, heat on a hot water bath for an hour, and set aside to cool. If the color is deep purple, dilute the solution to a definite volume. Remove an aliquot and match against a standard manganese solution in Nessler jars or in a colorimeter. Compute the results as percentage of manganese (Mn or Mn_2O_3). (A series of manganese standard solutions are prepared from reagent (C) by removing aliquots of the manganous sulfate solution and developing the manganese color with potassium periodate in the same way as the solution of the sample.)

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 67.

REPORT ON FEEDING STUFFS.

By W. F. STERLING (Food, Drug and Insecticide Administration),
Washington, D. C.), *Referee*.

The reports of the associate referees follow and a duplication of their summaries is unnecessary.

The recommendations of the associate referees are approved¹.

The referee recommends—

(1) That the toluene distillation method for moisture be made official, final reading.

(2) That the referee send out samples of hoof meal mixtures for preliminary collaborative work and get suggestions and criticisms of the hoof meal method as presented.

REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (Department of Agriculture, Harrisburg, Pa.),
Associate Referee.

During the past year collaborators interested in the composition of feeding stuffs continued the study of the detection of dried buttermilk in feeds. The referee submitted a modification of the method previously presented² in which the presence of buttermilk was determined by identifying, microscopically, the *Bacillus lactis* stained by methylene blue.

In the modified method it was suggested that the bacteria be stained by the Gram method in order to accentuate the *B. lactis*, which are Gram positive. In addition to this change the analysts were instructed to isolate the bacteria from the milk particles by triturating the feed in a mortar with water. The method is as follows:

PREPARATION OF SOLUTIONS FOR STAINING
GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA.

Sterling's Gentian Violet Solution.

	grams
Gentian Violet.....	4
Aniline Oil.....	2
Alcohol.....	10
Water.....	88

Iodine Solution.

Iodine.....	1
Potassium Iodide.....	2
Water.....	300

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 67.

² *This Journal*, 1928, 11: 36.

Carbol-Fuchsin Solution (Concentrated)

	grams
Fuchsin	1
Phenol.....	5
C ₂ H ₅ OH.....	10
Water.....	85

Diluted Carbol-Fuchsin Solution.

Carbol-Fuchsin Concentrated	20
Water.....	100

PREPARATION AND EXAMINATION OF SAMPLES.

Place 10 grams of dried buttermilk in a porcelain mortar and triturate thoroughly with pressure. Add a small quantity of water to form a pasty mass and continue the trituration with pressure, gradually adding water until the total volume becomes 100 cc. Allow the solid matter to settle out.

After the mixture has stood for 15 minutes, place a small drop from the surface of the supernatant liquid on a glass slide, using a glass rod or pipet. After spreading, place the preparation in a warm place upon a level surface to allow for drying within 5-10 minutes. After drying, dip the slide in xylol or gasoline for 1 minute, drain, and dry. Next immerse the preparation in 90 per cent alcohol and dry again.

Stain the smear several seconds with Sterling's gentian violet solution and wash with water. Follow with the iodine solution, staining several seconds, and again rinse in water. Follow with acetone, again washing with water. Lastly, stain with diluted carbol-fuchsin solution and wash with water. Dry between a folded filter paper and "fix" by heating in a low gas flame or on a hot plate. Examine for *B. lactis*, using the oil immersion lens or possibly the next lower magnification.

The collaborators were requested to apply the method to samples of buttermilk and to feeds with and without buttermilk present. Reports were received from eight laboratories, and the results are summarized as follows:

1.—*B. lactis* were found in all the various samples of buttermilk examined. In most instances the bacteria were very abundant, but if the specimen represented a "cultured" product, few *B. lactis* were present.

2.—Examination of ingredients used in stock feeds showed that many of them contained bacteria which might be confused or mistaken for *B. lactis*.

3.—Reports on the detection of buttermilk which had been added to feeds in various amounts indicated that the analysts were able to detect it down to 0.1 per cent, but the results were doubtful when the amount present was less than 2 per cent. Several of the collaborators indicated that 5 per cent of buttermilk could be detected with certainty.

From the results observed it is quite evident that the modified method has not met the success of the method tentatively adopted last year owing, largely, to the fact that in the latter method actual particles of buttermilk were identified by the *B. lactis* enclosed within their

areas. The danger of confusing various types of bacteria was thus avoided.

It is recommended¹, therefore, that for the present no change be made in the method adopted tentatively last year.

REPORT ON MINERAL MIXED FEEDS.

By H. A. HALVORSON (State Dairy and Food Department, St. Paul, Minn.), *Associate Referee*.

During the past year it was not possible for the Associate Referee on Mineral Mixed Feeds to carry out the recommendations of this association with reference to the study of the proposed method for lime (CaO) in mineral feeds and the proposed method for the determination of iodine in mineral feeds. The plan was to make up samples of known composition to send to collaborators for the determination of lime and iodine by the proposed methods and modifications of these methods suggested by collaborators. An additional year of experience on official samples of mineral feeds indicates the advisability of continuing the work along the lines outlined in the previous reports to the association.

RECOMMENDATIONS¹.

It is recommended—

(1) That the proposed method for the determination of lime in mineral feeds² be further studied and that the acetic acid modification of this method³ be tried for comparison of results.

(2) That the proposed method for the determination of iodine in mineral feeds published in the 1926 report² be further studied and that consideration also be given to other proposed methods.

REPORT ON MOISTURE IN FEEDING STUFFS.

By G. E. GRATTAN (Department of Agriculture, Ottawa, Can.),
Associate Referee.

During the year the referee carried on some investigation work, using a temperature of 135°C. Owing to some suggestions which were received after the work was completed, it was thought advisable not to make a report at this meeting. It is recommended that the work be continued for another year¹.

A paper, entitled "Determination of Hoof Meal", was presented by W. F. Sterling. It has been published⁴.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 67.

² *This Journal*, 1927, 10: 174.

³ *Ibid.*, 1928, 11: 158.

⁴ *This Journal*, 1929, 12: 129.

REPORT ON SUGARS AND SUGAR PRODUCTS.

By R. T. BALCH (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

During the current year active work was conducted by the Associate Referees on Honey; on Drying, Densimetric and Refractometric Methods; on Polariscopic Methods; and on Chemical Methods for Reducing Sugars. Progress reports from these subdivisions will be presented in order. The Associate Referee on Maple Products was unable to complete his work, hence submitted no report. The association was again unable to find anyone willing to undertake the work relating to starch conversion products, but it is hoped that active work may be started during the coming year, since starch conversion products are being used in increasing quantities each year, and the methods of analysis for these products are far from satisfactory.

Regarding the investigations being conducted by the other subdivisions, the referee believes that they are being carried out in a most efficient manner and that the program of work which will be recommended is very essential to the best interests of the association. The referee, therefore, concurs in these recommendations.

REPORT ON HONEY.

By H. A. SCHUETTE (Department of Chemistry, University of Wisconsin, Madison, Wis.), *Associate Referee.*

The suggestion was made in 1924 by Auerbach and Bodländer¹ that the addition to honey of commercial invert sugar, made from sucrose by enzyme conversion, would affect the relative proportion of levulose and dextrose which normally obtains in the former to such an extent as to cause a deviation from a definite minimum. This minimum for German honeys was set by them at 1.06. Gronover and Wohnlich² subsequently took exception to this ratio, a position in which Fiehe³ later concurred.

The establishment of this ratio rests upon a convenient method for the determination of one sugar in the presence of another and the determination of the other "by difference" after the total reducing sugars as invert have been obtained in a fresh sample. Auerbach and Bodländer chose to adapt to honey analysis an older method, the salient feature of which consists of the iodometric oxidation of dextrose to gluconic acid without a concurrent effect upon the levulose. These ends appear to be accomplished if the reaction is carried out in a solu-

¹ *Z. Nahr. Genussm.*, 1924, 47: 233.

² *Ibid.*, 48: 405.

³ *Z. Untersuch. Lebensm.*, 1926, 52: 244.

tion made weakly alkaline by the addition of a sodium carbonate-sodium bicarbonate mixture.

This method was submitted last year to a preliminary study¹, during the course of which data were obtained which seemed to point to the desirability of setting a definite time limit to the action of iodine on aldose sugars if the results were to be reproducible by others. Two hours was the suggested reaction period. It was also indicated within the compass of the investigation, which was not so extensive as to warrant very definite conclusions, that Auerbach and Bodländer's 1 : 1.06 ratio, when applied to American-grown honeys, seemed to possess merit as indicative of the presence of artificial invert sugar of biological origin.

This investigation was continued along lines previously followed by the associate referee and made the subject of collaborative study last year. Seven honeys, which were obtained from growers in five states, served as experimental material (Table 1); with the exception of one,

TABLE 1.
Analysis of honeys.

SOURCE OF HONEY	WATER*	ASH*	POLARIZATION*	DEXTRIN†	SUCROSE‡
	<i>per cent</i>	<i>per cent</i>	<i>° V.</i>	<i>per cent</i>	<i>per cent</i>
Alabama	14.16	0.53	+ 2 5	13 80	1 64
California (orange blossom)	16.21	0 06	-13 5	1 55	3 85
Kansas (sweet clover)	17.60	0 11	-14 3	1 55	1 77
Missouri (Spanish needle)	16.69	0 11	-15 5	1.55	0.57
Wisconsin I	15.18	0.06	-14.5		2.38
Wisconsin II	13.05	0 03	-11.3		4.01
Wisconsin III (clover)	14.17	0 11	- 3 4		2.19

* Analyses by Vera Templin.

† Analyses by Jennette N. Terrill.

‡ Analyses by Philippa G. Gilchrist.

which is apparently of "honey dew" origin, they fell well within the requirements for legal honey. The dextrose and levulose content of an eighth honey, the analysis of which was not obtained but which had been submitted as genuine by a honey growers' association, was determined by one of the collaborators and included in the group.

The levulose-dextrose ratio (Table 2) of these honeys was determined in the laboratory of the associate referee by Miss Philippa G. Gilchrist as collaborator, who carried out the oxidations of the dextrose at a thermostatically controlled temperature of 20°C. Then to two of this number, which were clover honeys from Kansas and Wisconsin, there

¹ *This Journal*, 1928, 11: 104.

TABLE 2.

Dextrose and levulose content of honeys obtained from various sources.

SOURCE OF HONEY	ANALYST	DEXTROSE <i>per cent</i>	LEVULOSE <i>per cent</i>	REDUCING SUGARS AS INVERT <i>per cent</i>	LEVULOSE- DEXTROSE RATIO
Alabama	1	31.42	34.41	65.83	1 : 1.09
California (orange blossom)	3	36.63	37.17	73.80	1 : 1.02
Colorado (alfalfa)	3	37.92	40.92	78.85	1 : 1.08
Kansas (clover)	1	35.77	38.22	74.00	1 : 1.07
	2	35.41	38.00	73.41	1 : 1.07
	3	35.52	38.72	74.24	1 : 1.09
Missouri (Spanish needle)	3	31.33	38.20	69.53	1 : 1.22
Wisconsin I (clover)	1	35.30	39.47	74.77	1 : 1.12
	2	34.97	39.59	74.56	1 : 1.13
	3	36.63	39.23	75.86	1 : 1.07
Wisconsin II (clover)	3	34.97	40.23	75.20	1 : 1.15
Wisconsin III (clover)	3	35.39	39.18	74.57	1 : 1.10

1. L. D. Hammond.

2. E. O. Huebner.

3. Philippa G. Gilchrist.

was added a commercial invert sugar sirup (levulose 31.13 per cent, glucose 42.21 per cent) in the amounts of 10 and 25 per cent, respectively. These mixtures, together with the original unaltered honeys, were sent out to the collaborators with the request that they determine (1) the total reducing sugars as invert by the method of Munson and Walter¹, (2) the dextrose volumetrically by the method of Auerbach and Bodländer, and (3) levulose by difference. With respect to the determination of the dextrose, it was suggested that 2-gram samples be transferred in duplicate with water to 250 cc. volumetric flasks and that 1 cc. of alumina cream be added before dilution to volume. Of the filtered solution 25 cc. was to be oxidized with the reaction period held rigidly at 2 hours. A criticism of the procedure was requested, together with a statement of conclusions drawn from the levulose-dextrose ratio in so far as a deviation from the value 1.06 might indicate the presence of added invert sugar. Data recorded in Table 3 are pertinent to this phase of the study.

COMMENTS OF COLLABORATORS.

E. O. Huebner, Madison, Wisc.: The glucose-fructose ratio as obtained would indicate that samples Nos. 1, 2, 3, and 4 come within the range for pure honeys, whereas samples Nos. 5 and 6 indicate adulteration to the extent of less than 10 per cent of commercial invert sugar.

¹ *Methods of Analysis*, A. O. A. C., 1925, 190.

TABLE 3.
Analysis of experimental honeys.

	ANALYTICAL DATA	
	L. D. HAMMOND	E. O. HUEBNER
	<i>per cent</i>	<i>per cent</i>
Sample 2		
dextrose	35.75	35.44
levulose	39.60	39.22
levulose/dextrose	1.11	1.11
Sample 3		
dextrose	36.17	35.99
levulose	38.66	38.45
levulose/dextrose	1.07	1.07
Sample 5		
dextrose	35.92	36.13
levulose	38.87	37.26
levulose/dextrose	1.08	1.03
Sample 6		
dextrose	36.67	36.15
levulose	37.79	37.30
levulose/dextrose	1.03	1.03

In making the honey solution, 4-gram samples were taken and made to a volume of 500 cc. Since small differences in the weight of the sample greatly influence final results it was felt that greater accuracy could be obtained with the larger sample. The concentration of sugars remains the same as with the suggested solution. The determinations of invert sugar and glucose were carried out according to the notes submitted to the collaborators. For the glucose oxidation 25 cc. of the iodine solution was used.

Two suggestions that I believe would increase the value of the iodometric method for glucose in honey are: (1) to double the volume of the solution to be oxidized, and (2) to standardize the quantity of iodine solution to be added. When the suggested method is used, it will be noted that a difference in the titration of 0.1 cc. of sodium thiosulfate accounts for a difference of 0.45 per cent in the glucose content. By doubling the volume of sample this error could be materially reduced. As the percentage of glucose obtained varies with the time allotted for oxidation it is not unreasonable to suppose that it would also vary with the quantity of iodine present. In fact, experiments on some of these samples bear out this suspicion.

Huebner carried out his oxidations "at room temperature".

L. D. Hammond, Washington, D. C.: These results show all the samples except No. 6 to be within the limits of the 1 : 1.06 ratio proposed for pure American honey.

Hammond stated further that he had carried out his oxidations and volumetric measurements at 20°C., and that had time permitted he would have studied the effect on this reaction of temperatures other than the one prescribed. The use of Monroe, rather than the ordinary Gooch crucibles, gave better duplicates, he found, in the determination of total reducing sugars.

DISCUSSION.

That a deviation from the tentative levulose-dextrose ratio of 1.06 did not reveal the presence of added invert sugar sirup was the unanimous

opinion of the collaborators with respect to three samples. They were divided as to conclusions with respect to a fourth, when as a matter of fact but two of this group were pure honeys. In but one instance (sample 5) did the calculated and determined quotients agree, and in another (sample 6) the discrepancy was 0.05, although the general conclusions were correct.

The element of uncertainty which surrounds the results of this year's study raises the question of the validity of the Auerbach-Bodländer hypothesis expressed earlier in this report. Were the manufacture of commercial invert sugar standardized to the point where a product uniform in its content of levulose and dextrose is produced, or were commercial invert sugar in fact the 50-50 mixture of monosaccharides which theory demands—in the work done by the associate referee last year a product approaching closely the ideal was used as adulterant—the validity of interpreting the levulose-dextrose ratio in terms of purity of sample might be justified.

It may be timely to recall at this point that in Germany little value is placed upon the levulose-dextrose ratio¹ as the sole means of passing judgment upon a suspected honey, for in normal honeys this relationship has been found to vary, as König and Karsch² report, between 72.5 and 109; and, as Sieben² found, even as high as 131.8. The findings of Browne³, whose levulose values were calculated from the change in rotation with increased temperature, are also pertinent. His average levulose and dextrose value for 99 honeys when interpreted in this manner is 1.19, and instances are not wanting in his data of honey from certain floral nectars showing 1.32 and 1.96 ratios, respectively. In the experiences of the associate referee, data for which are not formally presented here, several honeys showed a levulose-glucose ratio less than unity, although there appears to be no valid reason for condemning the honeys in question.

In view of the results obtained and the comments given it would appear that no useful advantage obtains at the present time in attempting to establish a levulose-dextrose ratio as an index of the presence of added invert sugar sirup. Not until the matter can be intelligently approached from the other direction, that is until a satisfactory method is found for determining levulose in the presence of dextrose, can it be stated with finality that hypiodite solutions are selective in their oxidation of dextrose in instances of this kind. Hinton and Macara⁴ found that levulose is slightly affected.

¹ Fiehe. *Z. Untersuch. Lebensm.*, 1926, 52: 255

² *Ibid.*, 256.

³ U. S. Dept. Agr. Bur. Chemistry Bull. 110, 1908.

⁴ *Analyst*, 1924, 49: 23.

RECOMMENDATIONS¹.

It is recommended that attempts to establish a workable levulose-dextrose ratio that can be applied as an index of purity to honey without injustice to the legal yet seemingly abnormal honey be temporarily abandoned for critical studies of methods for determining dextrose on the one hand, and levulose on the other when they occur in the presence of one another, as in honey. A method claimed to be selective towards levulose is that of Nyn², studies of which were recommended last year. This study is in progress in the laboratory of the associate referee and, it is understood, elsewhere.

No report on maple products was given by the associate referee.

No report on starch conversion products was given. No associate referee was appointed.

REPORT ON DRYING, DENSIMETRIC AND REFRACTOMETRIC METHODS.

By J. F. BREWSTER (Bureau of Standards, Washington, D. C.),
Associate Referee.

Previous collaborative work of this association has shown that the official drying, densimetric and refractometric methods for the determination of moisture (or dry substance) do not afford concordant results in the hands of different analysts for any but very high-grade sugar products. It was found, as a rule, that more satisfactory results were obtained with beet than with cane products. With regard to the latter the general statement may be made that high-grade materials, such as white and good raw sugars, first cane sirups and some of the refinery sirups, yield more acceptable results than the low-grade products, such as the second and third sugars of the raw sugar factory, while the various kinds of molasses offer a baffling problem.

Previous collaborative studies of the direct drying methods, especially with cane products, have given discouraging results. The papers of Brown, Sharp and Nees³ and of Rice⁴ upon this subject, published during the past year, state that what is termed "moisture" in impure sugar products is a matter of definition and as determined by any of the official methods is the result of certain definite conditions of analysis.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 68.

² *La Sucrerie Belge*, 1924, 44: 210.

³ *Ind. Eng. Chem.*, 1928, 20: 945.

⁴ *Ibid.*, *Anal. Ed.*, 1929, 1: 31.

This statement may apply to the densimetric and refractometric methods as well, and there is little doubt that discrepancies among the results of different operators when the results of the individual operators closely agree among themselves is due chiefly to what would appear to be the impossibility of duplicating conditions. Sandera¹ states that none of the methods so far employed for the determination of dry substance in sugar products will give absolute results, but that the refractometric method is to be preferred. This statement perhaps summarizes the opinion of most investigators.

The recently published results of Brown, Sharp and Nees were obtained with a drying method applied to high purity beet sirup in which painstaking attention to details was observed. The results were in excellent agreement, but the authors point out that this procedure is too time-consuming for any but special cases, in which the effort to obtain close results is justified. They do not recommend their procedure as being applicable to cane products.

The method of determining moisture by distillation with toluene, as applied to substances other than sugar products by Bidwell and Sterling², has never been subjected to study by this association. Rice, who applied this method with the addition to the distillation mixture of diatomaceous earth, reported satisfactory results in the analysis of refinery sirup containing a high percentage of reducing sugars. It would seem, however, that this method would be open to the same criticism as the other drying methods, in which so much depends upon the composition of the product and the exact conditions of analysis.

In the present status of the methods for the determination of moisture in sugar products it has been suggested that a study be made of the refractometric method, with the end in view of finding a procedure that may yield concordant results in the hands of different operators.

RECOMMENDATIONS³.

It is recommended—

- (1) That studies of these methods be continued.
- (2) That the refractometric method receive particular attention—
 - (a) by a survey of the tables of refractive indices corresponding to sucrose concentration; and
 - (b) by a study of the influence of known quantities of impurities upon the refractive index of sucrose solutions.

¹ *Z. Zuckerind. czechoslovak. Rep.*, 1928, 53, 1.

² *This Journal*, 1925, 8: 295.

³ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 68.

REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*.

In the report of the associate referee for last year¹ it was recommended that the four inversion methods used previously be "applied to mixtures of pure sugars with pure amids and amino acids". Experiments along this line were made and are reported below.

The second recommendation advised that "the isolation in the pure state of the amids and amino acids occurring in cane black strap be attempted". In a previous investigation by the writer² it had been found that the amids present in fresh sugar cane juice can be easily isolated in crystalline form, and that the greater part of them consists of asparagine. The same methods of preparation were tried with a Porto Rican blackstrap, but without success. It appears that the large quantities of colloidal matter in molasses prevent the crystallization of the amids. Even treatment of the solutions of the amids, liberated from their mercury compounds by means of hydrogen sulfide, with activated carbon proved unsuccessful. Vondrák's method for determining amids in beet products³, used by Eisenschimmel⁴ for the isolation of glutamin, also gave unsatisfactory results. It became evident that the preparation of amids and amino acids from cane blackstrap presents a research problem of such character that it can be better undertaken as an independent investigation than as a project of this association.

The third recommendation in last year's report was to study the effect of lead clarification on the results of Clerget determinations in sugar products. Experiments were made along this line also, but two chemists working in one laboratory obtained such divergent results that it was decided to postpone this investigation until the research work on products not requiring lead clarification has been completed.

The analytical work proposed in the first recommendation was carried out in the Carbohydrate Division of the Bureau of Chemistry and Soils by Victor Birckner, and in this laboratory by C. A. Gamble and J. E. Mull; the writer wishes to express to them his appreciation of their collaboration. The working plan adopted by the referee and sent to the collaborators was as follows:

The products to be used in this year's investigation are—

A. Sucrose (Domino tablet sugar).

B. Invert sugar sirup made by means of invertase, of 56.2 Brix by refractometer, and adjusted to pH 7.

¹ *This Journal*, 1928, 11: 175.

² *Orig. Com. 8th Intern. Congr. Appl. Chem.*, 1912, 8: 103.

³ *Z. Zuckerind. czechoslovak. Rep.*, 1926/27, 51: 261.

⁴ *Ibid.*, 337.

C. A commercial invert sugar sirup, prepared by diluting Nulomoline (kindly furnished by N. Fuad of the Nulomoline Company) to 60 Brix by refractometer, and adjusted to pH 7.

D. A solution containing in 100 cc. total volume 2.6 grams of 1-aspartic acid plus the required quantity of sodium hydroxide to bring the pH of the solution to 7.

E. A solution containing in 100 cc. total volume 2.6 grams of 1-asparagine plus the required quantity of sodium hydroxide to bring the pH to 7.

Use the concentrations given below for both the direct and invert polarization:

1. Sucrose, 13 grams to 100 cc.
2. Sucrose, 13 grams, plus 10 cc. of solution D, to 100 cc.
3. Sucrose, 13 grams, plus 10 cc. of solution E, to 100 cc.
4. Sirup B, 23.132 grams (= 13 grams solids) to 100 cc.
5. Sirup B, 23.132 grams, plus 10 cc. of solution D, to 100 cc.
6. Sirup B, 23.132 grams, plus 10 cc. of solution E, to 100 cc.
7. Sirup C, 21.667 grams (= 13 grams solids) to 100 cc.
8. Sirup C, 21.667 grams, plus 10 cc. of solution D, to 100 cc.
9. Sirup C, 21.667 grams, plus 10 cc. of solution E, to 100 cc.
10. Mixture of 6.5 grams sucrose and 11.566 grams sirup B, to 100 cc.
11. Mixture of 6.5 grams sucrose, 11.566 grams sirup B, and 10 cc. of solution D, to 100 cc.
12. Mixture of 6.5 grams sucrose, 11.566 grams sirup B, and 10 cc. of solution E, to 100 cc.
13. Mixture of 6.5 grams sucrose and 10.833 grams sirup C, to 100 cc.
14. Mixture of 6.5 grams sucrose, 10.833 grams sirup C, and 10 cc. of solution D, to 100 cc.
15. Mixture of 6.5 grams sucrose, 10.833 grams sirup C, and 10 cc. of solution E, to 100 cc.

Use the same inversion methods as last year, viz., official invertase method, A. O. A. C. acid method, and Jackson and Gillis methods No. II and IV. Allow all the solutions, for the direct as well as for the invert reading, to stand overnight, at 26°-27°C. Make the readings at 20C.° in a water-jacketed tube. The same Clerget factors are to be used as last year, and they are to be based on the total sugar concentration.

The tables showing the results of the analyses are arranged as in previous reports. The percentage figures for the experiments with sucrose are given on the basis of 26 grams in 100 cc.; for the other products the results are expressed in percentage of total sugars.

When the mixture of sucrose and sirup B was first analyzed in this laboratory, it became evident that the sirup still contained some active invertase, in spite of the fact that the sirup had been heated to 80°C. in the presence of activated carbon. In the meantime a portion of this sirup had already been shipped to Washington. Both portions were reheated to destroy the invertase, but there was evidently a difference in the manner of heating in the two laboratories, because the analytical results were widely apart. For this reason they are not given in the tables, and this part of the work should be repeated next year.

The agreement between the results obtained in the two laboratories is not always so good as might be desired, owing no doubt to the lower quality of the sucrose used in part of the work in one of the two laboratories. Nevertheless, the influence of the amino compounds is so pro-

TABLE 1.

Sucrose.

ANALYST, AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
Invertase method			
Victor Birckner	49 88	-15 89	49 79
C. A. Gamble, J. E. Mull	49.95	-16.03	49 95
			49.87
A. O. A. C. acid method			
Victor Birckner	49 88	-16.64	49 94
C. A. Gamble, J. E. Mull	49 95	-16 58	49 95 ¹
			49 95
Jackson and Gillis method No. II			
Victor Birckner	49 65	-16 90	49 91
C. A. Gamble, J. E. Mull	49.70	-16 88	49 93
			49 92
Jackson and Gillis method No. IV			
Victor Birckner	49 60	-16 64	49 94
C. A. Gamble, J. E. Mull	49 68	-16.58	49 96
			49 95
<i>Sucrose plus aspartic acid.</i>			
Invertase method			
Victor Birckner	49 63	-16 17	49 81
C. A. Gamble, J. E. Mull	49 70	-16 28	49 95
			49 88
A. O. A. C. acid method			
Victor Birckner	49 63	-16 28	49 48
C. A. Gamble, J. E. Mull	49 70	16 18	49 46
			49 47
Jackson and Gillis method No. II			
Victor Birckner	49 54	-17.08	49.96
C. A. Gamble, J. E. Mull	49 60	-17 05	49 98
			49 97
Jackson and Gillis method No. IV			
Victor Birckner	49.48	-16 28	49 58
C. A. Gamble, J. E. Mull	49.58	-16 18	49 58
			49 58
<i>Sucrose plus asparagine.</i>			
Invertase method			
Victor Birckner	49 79	-16.09	49 87
C. A. Gamble, J. E. Mull	49 85	-16 10	49 92
			49.90
A. O. A. C. acid method			
Victor Birckner	49.79	-16 18	49 53
C. A. Gamble, J. E. Mull	49.85	-16.13	49 53
			49 53
Jackson and Gillis method No. II			
Victor Birckner	49 67	-16 93	49 95
C. A. Gamble, J. E. Mull	49.65	-16.95	49.95
			49.95
Jackson and Gillis method No. IV			
Victor Birckner	49.56	-16.18	49 57
C. A. Gamble, J. E. Mull	49.63	-16.13	49 58
			49 58

TABLE 2.

Sirup C.

ANALYST, AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
Invertase method			
Victor Birekner	-12 79	-13 73	1 42
C. A. Gamble, J. E. Mull	-12 73	-13 70	1 47
			1 45
A. O. A. C. acid method			
Victor Birekner	-12 79	-15 25	3 69
C. A. Gamble, J. E. Mull	-12 73	-15 23	3 75
			3 72
Jackson and Gillis method No. II			
Victor Birekner	-13 68	-15 55	2 80
C. A. Gamble, J. E. Mull	-13 65	-15 50	2 77
			2 79
Jackson and Gillis method No. IV			
Victor Birekner	-13 22	-15 25	3 06
C. A. Gamble, J. E. Mull	-13 13	-15 23	3 17
			3 12

Sirup C plus aspartic acid.

Invertase method			
Victor Birekner	-13 09	-13 88	1 20
C. A. Gamble, J. E. Mull	-13 01	-13 90	1 35
			1 28
A. O. A. C. acid method			
Victor Birekner	-13 09	-14 92	2 75
C. A. Gamble, J. E. Mull	-13 01	-14 90	2 84
			2 80
Jackson and Gillis method No. II			
Victor Birekner	-13 76	-15 62	2 79
C. A. Gamble, J. E. Mull	-13 78	-15 70	2 88
			2 84
Jackson and Gillis method No. IV			
Victor Birekner	-13 50	-14 92	2 14
C. A. Gamble, J. E. Mull	-13 55	-14 90	2 04
			2 09

Sirup C plus asparagine.

Invertase method			
Victor Birekner	-12 94	-13 73	1 20
C. A. Gamble, J. E. Mull	-13 00	-13 78	1 18
			1 19
A. O. A. C. acid method			
Victor Birekner	-12 94	-14 95	3 02
C. A. Gamble, J. E. Mull	-13 00	-14 90	2 85
			2 94
Jackson and Gillis method No. II			
Victor Birekner	-13 68	-15 58	2 85
C. A. Gamble, J. E. Mull	-13 68	-15 65	2 95
			2 90
Jackson and Gillis method No. IV			
Victor Birekner	-13 42	-14 95	2 31
C. A. Gamble, J. E. Mull	-13 53	-14 90	2 07
			2 19

nounced that valid conclusions can safely be drawn. Aspartic acid or asparagine, when added to sucrose alone, evidently has no effect on the sucrose determination by the invertase method or by Jackson and Gillis method No. II, in neither of which do the inverted solutions contain strong acid. But both the plain acid method and Jackson and Gillis method No. IV, where the inverted solutions are strongly acid, give results very much too low. This is due to the fact that the two amino compounds show high dextrorotation in strongly acid solution, but only slight rotation, either positive or negative, in mildly acid or neutral solution.

The results obtained with the commercial invert sugar sirup alone confirm those found for similar sirups in 1924 and 1925¹. The two Jackson and Gillis methods give higher results than the invertase method, owing to the hydrolysis of reversion products by the hydrochloric acid; again method No. II shows a tendency to give somewhat lower results than No. IV, for reasons discussed in previous reports. The plain acid method yields values even higher than the Jackson and Gillis methods, on account of the effect of the free hydrochloric acid on the rotation of the levulose, without similar compensation in the solution for the direct reading.

In the series of tests where aspartic acid or asparagine was added to the sirup, the invertase method gave slightly lower results than for the sirup alone. Whether these deviations are accidental or actual will have to be determined by further work. It might be suggested that the discrepancies are caused by the effect of the slight difference in reaction between the uninverted and inverted solutions, but if this were so similar discrepancies should have been found in the experiments with sucrose plus aspartic acid or asparagine. However, in those tests very good agreement was obtained, and the reason for the deviations in the sirup series must be sought elsewhere.

Jackson and Gillis method No. II gives the same results in the presence of aspartic acid or asparagine as it does in their absence, for reasons already explained in discussing Table 1. Jackson and Gillis method No. IV, however, yields values much lower than No. II, because here the high dextrorotation of the amino compounds in a strongly acid medium exerts its influence. The plain acid method shows about the same figure as Jackson and Gillis method No. II, owing to the combined effect of reversion products, of amino compounds, and of levulose in a strongly acid solution. It is further noted that the difference between the results of the plain acid method for sirup alone and of the plain acid method for sirup plus amino compounds is about the same as the corresponding difference for Jackson and Gillis method No. IV. In both cases this difference is caused by the high dextrorotation of the amino compounds in strongly acid solution.

¹ *This Journal*, 1925, 8: 396; 1926, 9: 171.

TABLE 3.
Mixture of sucrose and sirup C.

ANALYST, AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	SUCROSE FOUND <i>per cent</i>	SUCROSE CALCULATED ON BASIS OF SAME METHOD
Invertase method				
Victor Birckner	18 54	-14 82	50 51	
C. A. Gamble, J. E. Mull	18 53	-14 90	50 61	
			50 56	50 60
A. O. A. C. acid method				
Victor Birckner	18 54	-16 00	51 86	
C. A. Gamble, J. E. Mull	18 53	-15 95	51 77	
			51 82	51 80
Jackson and Gillis method No. II				
Victor Birckner	18 02	-16 12	51 36	
C. A. Gamble, J. E. Mull	18 00	-16 20	51 30	
			51 33	51 32
Jackson and Gillis method No. IV				
Victor Birckner	18 15	-16 00	51 50	
C. A. Gamble, J. E. Mull	18 29	-15 95	51 63	
			51 57	51 51
<i>Mixture of sucrose and sirup C, plus aspartic acid.</i>				
Invertase method				
Victor Birckner	18 29	-15 09	50 48	
C. A. Gamble, J. E. Mull	18 28	-15 10	50 48	
			50 48	50 52
A. O. A. C. acid method				
Victor Birckner	18 29	-15 59	50 87	
C. A. Gamble, J. E. Mull	18 28	-15 53	50 77	
			50 82	50 87
Jackson and Gillis method No. II				
Victor Birckner	17 89	-16 33	51 33	
C. A. Gamble, J. E. Mull	17 88	-16 28	51 24	
			51 29	51 39
Jackson and Gillis method No. IV				
Victor Birckner	17 95	-15 59	50 58	
C. A. Gamble, J. E. Mull	17.95	-15 53	50 49	
			50 54	50 63
<i>Mixture of sucrose and sirup C, plus asparagine.</i>				
Invertase method				
Victor Birckner	18.44	-14.98	50 60	
C. A. Gamble, J. E. Mull	18 38	-15 03	50.58	
			50 59	50 49
A. O. A. C. acid method				
Victor Birckner	18.44	-15.58	51 04	
C. A. Gamble, J. E. Mull	18 38	-15.55	50 95	
			51.00	51 00
Jackson and Gillis method No. II				
Victor Birckner	17.94	-16.29	51 34	
C. A. Gamble, J. E. Mull	17 95	-16 35	51 45	
			51 40	51 40
Jackson and Gillis method No. IV				
Victor Birckner	18 03	-15.58	50.68	
C. A. Gamble, J. E. Mull	17 98	-15 55	50 56	
			50 62	50 67

In Table 3 the results in each case agree closely with those calculated always on the basis of the same method from the figures obtained for the components of the mixture. Again the value found by the invertase method is a little lower in the presence of aspartic acid, but the asparagine shows no such effect in this series. Jackson and Gillis method No. II gives in every instance results higher than the invertase method, because the hydrolyzed reversion products appear as sucrose, but the apparent sucrose figure found by this method is the same in the presence or absence of amino compounds, because the pH before and after inversion is the same. Jackson and Gillis method No. IV in the absence of amino compounds yields too high results, on account of the hydrolysis of reversion products, but in the presence of amino compounds it gives about the same results as the invertase method, because here the influence of the reversion products is counterbalanced by the lowering effect of the amino compounds in the presence of strong acid. The plain acid method, in the absence of amino compounds, again gives the highest results of all the methods, for reasons already explained, but when amino compounds are present the results are much lower. Here the combined effect of reversion products, of amino compounds, and of levulose in strongly acid solution produces a value higher than that by invertase or by Jackson and Gillis method No. IV, but lower than that by Jackson and Gillis method No. II.

In last year's report the opinion was expressed that the hydrochloric acid used for inversion in Jackson and Gillis method No. II might cause partial hydrolysis of asparagine, and might thus affect the result obtained by this method, but this year's results do not bear this out.

A careful analysis of the figures reported in the tables shows that a comparison of the results obtained by the different methods may be used to get an idea of the quantity of reversion products and of aspartic acid or asparagine (which are known to be the principal amino compounds in cane products) in the materials analyzed. The difference between Jackson and Gillis method No. II and the invertase method furnishes an approximate measure, in terms of sucrose, of the reversion products which are hydrolyzed by hydrochloric acid at room temperature. In Table 3, 0.72 per cent apparent sucrose for the mixture alone, 0.87 per cent for the mixture plus aspartic acid, and 0.91 per cent for the mixture plus asparagine will be noted. The corresponding figures for the sirup itself, Table 2, are 1.34 per cent, 1.56 per cent, and 1.71 per cent, respectively, or, as should be expected, about twice as much as in the corresponding mixtures containing equal parts of sucrose and sirup solids. The three figures in either series should, theoretically, be the same, but close agreement cannot be expected, because the extent of the hydrolysis of the reversion products depends on many factors that are not under control. Nevertheless, a higher result by Jackson

and Gillis method No. II than by the invertase method clearly indicates the presence of easily hydrolyzed reversion products.

On the other hand, the difference between the results by Jackson and Gillis methods No. II and No. IV furnishes an approximate measure of the amino compounds present, again in terms of sucrose. Turning once more to Table 3, the equivalent of 0.76 per cent sucrose is found for the mixture with aspartic acid, and for the mixture with asparagine, 0.73 per cent. In Table 2 the equivalent of 0.75 per cent is found for sirup plus aspartic acid, and for sirup plus asparagine, 0.71 per cent. From Table 1 0.78 per cent and 0.74 per cent, respectively, are calculated. As the same quantities of aspartic acid and of asparagine were added in every case, the analytical results evidently give a very close measure of the amino compounds present, the individual results ranging only from 0.71 to 0.78 per cent, in terms of sucrose.

In last year's report it was suggested that differences in the sucrose results by the four Clerget methods investigated were due partly to hydrolysis of reversion products and partly to the behavior of amino acids and amids in media of differing acidity. This opinion has been confirmed, and the significance of the facts has been elucidated. If the conclusions reached in this year's work be applied to the analyses made last year, it is found that the decolorized and inverted blackstrap was practically free from readily hydrolyzable reversion products. The difference between the results by Jackson and Gillis method No. II and by the invertase method was only 0.1 per cent, and in the three mixtures the results by the two methods were identical within the limits of error. A comparison between Jackson and Gillis method No. II and method No. IV gives the equivalent of 0.68 per cent (one laboratory found 0.57 per cent) sucrose, present as amino compounds, in the decolorized and inverted blackstrap. The three to one mixture of sirup solids and sucrose showed 0.42 per cent (calculated 0.51 per cent), the one to one mixture, 0.26 per cent (calculated 0.34 per cent), and the one to three mixture, 0.13 per cent (calculated 0.17 per cent). The agreement between the found and calculated values is quite good.

Reviewing the work done on the different Clerget methods during the past two years, the following conclusions may be drawn:

- (1) In the analysis of complex mixtures resembling cane products and containing sucrose, invert sugar, reversion products, and asparagine or aspartic acid, the invertase method must be used to determine the actual sucrose content.

- (2) The sucrose result by Jackson and Gillis method No. II is increased by reversion products hydrolyzed under the conditions of the experiment, but it is not affected by the presence of asparagine or aspartic acid.

- (3) The sucrose result by Jackson and Gillis method No. IV is increased by the hydrolysis of reversion products in the same way as that by

method No. II, but it is lowered in the presence of asparagine or aspartic acid.

(4) The difference between the sucrose result by Jackson and Gillis method No. II and that by the invertase method gives an approximate measure of the reversion products hydrolyzed by hydrochloric acid under the conditions of the analysis.

(5) The difference between the sucrose result by Jackson and Gillis method No. II and that by No. IV gives an approximate measure of the asparagine or aspartic acid present.

(6) The plain acid method may give any kind of a result, depending on the relative proportions between the different constituents of the mixture analyzed.

RECOMMENDATION¹.

In view of the fact that it was found impossible this year to study the effect of amino compounds in mixture with pure invert sugar on sucrose determination by the various Clerget methods, it is recommended that this year's work be repeated, and that sucrose alone, pure invert sugar alone, and mixtures of the two, with and without the further addition of aspartic acid or asparagine, be used.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS.

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),
Associate Referee.

It is probably true that despite the large number of methods for the estimation of reducing sugars which have been proposed, the method employed in the great majority of analyses is the one devised by Munson and Walker. In this method the precipitated copper is collected on a filter and estimated by one of several ways. This association requires that the direct weighing of cuprous or cupric oxide or even metallic copper be confined to analyses of products of high purity. The direct weighing method, however, is frequently employed on crude substances, such as natural juices and even on such a crude substance as blackstrap molasses. In such cases the danger of obtaining a copper precipitate contaminated by both organic and inorganic impurities is very great. It is an insidious source of error and seldom gives evidence of its presence. In the analyses of all such products the true copper can be found only by electrolytic or titration methods. While the electrolytic method is laborious and requires elaborate and expensive equipment, the thio-sulfate titration method is simple, rapid and reliable, and the starch iodide end point is sharp and decisive. The difficulty which apparently

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 68.

prevents its extensive use is to be found in the present specifications for the preparation of the solution for titration¹. The nitric acid solution of the copper, freed from the oxides of nitrogen by boiling and treatment with bromine, is made alkaline with ammonia, and the excess of ammonia is evaporated off. It is this tedious evaporation of ammonia that renders the analysis unsatisfactory. Occasionally there appears a precipitate of partially dehydrated cupric hydroxide that redissolves in acetic acid with great difficulty. Moreover, under these specifications the acidity of the solution is difficult to regulate. Popoff² has shown the necessity of titrating in the presence of very low and definite acetic acid concentration. If the nitric acid is neutralized by sodium hydroxide instead of ammonia these difficulties are obviated. To the nitric acid solution, sodium hydroxide is added until a faint turbidity of cupric hydroxide appears. One or two drops of acetic acid clears this turbidity, and the solution is ready for titration. The whole operation of preparing the solution requires but a few periods of momentary attention. This method for titration of copper has been studied in great detail by Popoff, and in its application to sugar analysis by Dr. Goergen and the associate referee at the Bureau of Standards. It is therefore believed that further collaborative work is unnecessary, and it is recommended that sodium hydroxide be substituted for ammonia in the preparation of the copper solution for titration.

The volumetric method of Lane and Eynon in which methylene blue is employed as an internal indicator is, on account of its convenience, accuracy and rapidity, largely displacing the gravimetric methods for reducing sugar. This method was adopted at the 1925 meeting of this association as a tentative method³ and is being subjected to study. One outstanding objection to all volumetric methods is that the time of reaction is necessarily variable while the end point is being sought. The writer has adopted the practice of starting a stop watch at the moment of incipient boiling and noting the time required for the preliminary boiling and completed titration, with the purpose in mind of applying corrections for great variations in time. The rather surprising fact developed that variations from the normal of as much as 2 minutes produced almost negligible variations in the titration value. This preliminary study seemed so profitable that it is recommended that the effect of variations in time of reaction upon the volumetric determination of reducing sugar be subjected to further study.

In the specifications of Lane and Eynon's method, tentatively adopted, the method of titrating a solution of unknown concentration requires the addition of the sugar solution in 10 cc. portions until the copper

¹ *Methods of Analysis*, A. O. A. C., 1925, 191.

² Unpublished manuscript.

³ *This Journal*, 1926, 9: 35.

approaches exhaustion. It is believed that the wording is unnecessarily specific and tends to cause the analyst to overrun the end point. In the routine titration of large numbers of solutions at the Bureau of Standards the practice has been followed of adding the sugar solution rapidly in indefinite volumes in the usual manner of titration until the faintest perceptible blue remains, then adding the indicator and completing the titration. This alteration in procedure has been found so effective that the titration of a solution of unknown concentration is almost invariably as accurate as one of known concentration.

As recommended at the last meeting, the determination of levulose in the presence of other reducing sugars by a modification of Nyn's method has been subjected to further study. It has been found that the method is not perfectly selective, but that aldose sugars increase the precipitation of copper perceptibly. Thus, regardless of the concentration of levulose, 13 mg. of dextrose precipitates the same weight of copper as 1 mg. of levulose.

By analyzing known mixtures of levulose and dextrose it is possible to determine the reducing power of the dextrose and thus form a basis for applying the necessary correction. The determination of the glucose correction for an unknown mixture, however, involves a complicated and laborious calculation, for neither dextrose nor levulose can be determined until both are known simultaneously. The calculation, therefore, requires making a series of approximations until one pair of assumed dextrose and levulose values fits the experimental data. If a large number of analyses is required, the expenditure of time for calculation is prohibitive. Fortunately, it has been found possible to construct a "nomogram" from which the ratio of levulose to total sugar can be read directly. This diagram will be published in the near future in the *Bureau of Standards Journal of Research*.

The determination of levulose by a modification of Nyn's method is as follows:

REAGENTS.

Ost's solution.—Mix as required one volume of (a) and four volumes of (b). Add the copper solution to the alkali with vigorous agitation.

(a) *Copper sulfate solution*.—Dissolve 25.3 grams of pure copper sulfate crystals and make to 200 cc.

(b) *Alkali carbonate solution*.—Dissolve 312.5 grams of potassium carbonate crystals (K_2CO_3 , 1½ H_2O) in about 800 cc. of boiling water, and add with agitation 125 grams of potassium bicarbonate. Cool, make to 1 liter, and filter.

DETERMINATION.

Transfer 50 cc. of mixed Ost's solution to a 150 cc. Erlenmeyer flask. Add 20 cc. of a solution containing 30–70 mg. of levulose. Immerse the flask within 1 inch of the top in a water bath at 49°C. for 2½ hours. Collect the precipitated cuprous oxide on a Gooch crucible and wash flask and precipitate free from unreacted copper. Dissolve the cuprous oxide by pipetting 5 cc. of 1 : 1 nitric acid and wash into the original

reaction flask. Determine copper preferably by thiosulfate titration¹. (It is convenient to standardize the thiosulfate solution in terms of milligrams of levulose.)

RECOMMENDATIONS.

It is recommended—

(1) That the official volumetric thiosulfate method, *Methods of Analysis*, A. O. A. C., 1925, 37, p. 191, be revised as follows:

Standard thiosulfate solution.—Prepare a solution of sodium thiosulfate containing 19 grams of pure crystals in 1 liter. Weigh accurately about 0.2 gram of pure copper and place in a flask of 250 cc. capacity. Dissolve by warming with 5 cc. of a mixture of equal volumes of strong nitric acid and water. Dilute to 50 cc., boil to expel the red fumes, add a slight excess of strong bromine water, and boil until the bromine is completely driven off. Cool and add a strong sodium hydroxide solution with agitation until a faint turbidity of cupric hydroxide appears. Discharge the turbidity with a few drops of 80 per cent acetic acid and add 2 drops in excess. The solution should now occupy a volume of 50–70 cc. Add 10 cc. of 30 per cent potassium iodide solution. Titrate at once with the thiosulfate solution until the brown tinge becomes weak and then add sufficient starch indicator (p. 48, 3 (e)) to produce a marked blue coloration. Continue the titration cautiously until the color due to free iodine has entirely vanished. (Toward the end the blue color changes to a faint lilac. If at this point the thiosulfate is added drop by drop and a little time is allowed for complete reaction after each addition, no difficulty is experienced in determining the end point within a single drop.) 1 cc. of the thiosulfate solution = about 0.005 gram of copper.

(2) That par. 38, p. 192, last two lines, be revised to read as follows: under 37, beginning with "Cool and add strong sodium hydroxide solution, ———".

(3) That the last paragraph on p. 35, Vol. IX, 1926, of *This Journal* be amended to read as follows:

DETERMINATION.

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 cc. of Soxhlet's solution 15 cc. of the sugar solution and heat to boiling over a wire gauze. Boil about 15 seconds and add rapidly further quantities of the sugar solution until only the faintest perceptible blue color remains. Then add 2–5 drops of methylene blue and complete the titration by dropwise additions of sugar. (The results of this titration will, in general, be in error by not more than 1 per cent.)

COMMITTEES NAMED BY THE PRESIDENT.

Committee to Wait upon Secretary of Agriculture: A. G. McCall and L. D. Haigh.

Committee to Wait upon Honorary President: B. B. Ross and C. D. Howard.

Committee on Resolutions: W. W. Randall, C. A. Browne, and H. C. Lythgoe.

Committee on Auditing: J. W. Sale, W. W. Randall, and G. S. Jamieson.

Committee on Nominations: F. P. Veitch, B. B. Ross, and H. H. Hanson.

¹ *Methods of Analysis*, A. O. A. C., 1925, 191.

FIRST DAY.
MONDAY—AFTERNOON SESSION.

REPORT ON FERTILIZERS.

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.),
Referee.

The work on fertilizers has been progressing as planned. The Associate Referee on Phosphoric Acid has been studying the effect of sulfates on the volumetric estimation of phosphoric acid. Since several publications have called attention to the presence of sulfates as a source of error, it was considered necessary to ascertain whether any changes should be made in the methods of the association. The Associate Referee on Nitrogen has continued his work on methods for estimating inorganic nitrogen (nitrates and ammonia) in the presence of organic nitrogen, with quite satisfactory results. The Associate Referee on Nitrogen Activity in Fertilizers has secured satisfactory results in collaborative tests on the revised alkaline permanganate method proposed last year. The Associate Referee on Potash has studied the effect of acidity on the method for chlorides in fertilizers adopted provisionally last year and has found the acidity of fertilizer extracts to have little or no effect, though there may be some decrease owing to absorption or the presence of ammonia salts. Although the results secured are little lower than the calculated, the method seems to be satisfactory, and the associate referee should now be able to return to a study of methods for the estimation of potash.

The recommendations of the referee are given in the reports of the associate referees¹.

**THE VOLUMETRIC DETERMINATION OF PHOSPHORIC
ACID.**

By WM. H. ROSS (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

A volumetric method for the analysis of phosphates specifying ammonium molybdate as a precipitating agent was first proposed by Pemberton² in 1882. The method consists in adding ammonium nitrate to a nitric acid solution of the phosphate, heating to about 60°C., and titrating with a standard solution of ammonium molybdate until no further formation of a yellow precipitate occurs. This method was

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 69.

² *J. Franklin Inst.*, 1882, 113: 184.

modified in 1893 by treating the phosphate solution near the boiling point with an excess of ammonium molybdate, filtering, dissolving the washed precipitate in an excess of standard potassium hydroxide, and titrating the excess of alkali with standard acid, phenolphthalein being used as indicator.

This method received the prompt attention of the Referee on Phosphoric Acid, B. W. Kilgore, and the following year it formed the subject of a collaborative study of this association. This study was continued for the following four years, or until 1898, when it was adopted officially with some modifications as an alternative method for the determination of phosphoric acid. The more important of these modifications included a change in the temperature at which precipitation is made from near boiling to 45°–50°C. and in the strength of the molybdate solution from 66 grams of molybdenum trioxide per liter to 50 grams.

The referee's report for 1898 also made reference to tests on a further modification of the method which consisted in precipitating at room temperature with continuous stirring rather than at 45°–50°C. with occasional shaking. A favorable report on this modification of the volumetric method was made to the association two years later by Williams¹. This report and the satisfactory results obtained in the collaborative study of this modified procedure resulted in its adoption in 1900 as an alternative step in the optional volumetric method for determining phosphoric acid. The last of these reports, presented in 1903, gave results of a comparative study of this method and the gravimetric method.

Although no report on the volumetric method has thus been presented to this association in 25 years, numerous papers on the subject have appeared in the literature during this time. These publications show that the method has not been entirely reliable under all conditions and that slight deviations from the accepted procedure materially affect the results². The presence of sulfates in particular was found to be a disturbing factor since they produce high results when precipitation is made at 45°–50°C., the temperature most commonly used. This was first pointed out by Richardson³, who recommended the elimination of sulfates by the addition of the equivalent amount of a barium chloride solution immediately following the acid digestion of the sample, cooling, making up to volume, and filtering in the usual way. It was shown that the high results obtained in the presence of sulfates were due to the formation of a complex ammonium sulfomolybdate under the conditions of phosphoric acid precipitation by ammonium molybdate, which has the acid nature of ammonium phosphomolybdate. This was later confirmed by Falk

¹ U. S. Dept. Agr., Div. Chem. Bull. 62, p. 55 (1901).

² Cf. Carpenter. *J. Ind. Eng. Chem.*, 1910, 2: 157.

³ *J. Am. Chem. Soc.*, 1907, 29: 1314.

and Sugiura¹, who expressed the opinion that the ammonium sulfomolybdate combination is an essential part of the phosphomolybdate precipitate.

The removal of sulfates by the addition of a barium salt was also recommended by Breckenridge².

Shuey³ showed that the presence of sulfates does not interfere, and that their removal is unnecessary if precipitation is made at 20°–30°C. with stirring, as in the alternative official volumetric method. A similar conclusion was reached by Bible⁴ in a paper that appeared this year.

In view of these observations, a collaborative study of the relative accuracy of the two official procedures for the volumetric determination of phosphoric acid in the presence of sulfates was undertaken at the suggestion of the Referee on Fertilizers.

Two standard samples were selected for this work. Sample No. 1 was pure monopotassium phosphate, prepared by treating pure potassium carbonate with an equivalent amount of a highly purified sample of crystallized phosphoric acid, concentrating to crystallize out monopotassium phosphate, recrystallizing three times, and drying at 110°C. The crystals were finally ground in an agate mortar to go through a 100-mesh sieve.

Sample No. 2 was the Tennessee phosphate rock standard sample No. 56 of the Bureau of Standards.

The directions sent to the collaborators called for the analysis of these two samples with and without sulfates added, by (1) precipitating at 45°–50°C. as directed under paragraph 10 (a)⁵; and (2) precipitating at room temperature with stirring as directed under paragraph 10 (b).

DIRECTIONS FOR COLLABORATIVE WORK.

A. Determine total P_2O_5 in Sample No. 1 by the following procedures, using the reagents listed in the last edition of Methods of Analysis, page 3, section 8.

(1) Dry the sample at 105°C. for 1 hour. Dissolve 2.5 grams of the sample in water and dilute to 500 cc. Withdraw an aliquot of 10 cc., add 10 grams of ammonium nitrate, acidify with a few drops of nitric acid, dilute to 75–100 cc., heat in a water bath to 45°–50°C., and add 50 cc. of the molybdate solution. Allow the mixture to remain in the bath, stirring occasionally, for 30 minutes; decant at once through a filter; wash the precipitate twice by decantation with 25–30 cc. portions of water, agitating thoroughly; and allow to settle. Transfer the precipitate to the filter and wash with cold water until the filtrate from two fillings of the filter (9 cm.) yields a pink color upon the addition of phenolphthalein and one drop of the standard alkali (a). Transfer the precipitate and filter to the beaker or precipitating vessel, dissolve the precipitate in a small excess of the standard alkali (b), add about six drops of phenolphthalein indicator, and titrate with the standard acid (c).

¹ *J. Am. Chem. Soc.*, 1915, 37: 1507.

² *Ind. Eng. Chem.*, 1924, 16: 1180.

³ *J. Ind. Eng. Chem.*, 1917, 9: 367.

⁴ *This Journal*, 1928, 11: 126.

⁵ *Methods of Analysis*, A. O. A. C., 1925, 3.

(a) Different precipitates vary in the amount of washing required to remove acidity, and it is therefore important that tests be made of the washings from each precipitate, in order to avoid incomplete or excessive washing. Improper washing is perhaps one of the most frequent sources of error in the volumetric analysis of phosphates.

(b) Ammonia is evolved when the precipitate is dissolved in an excess of the standard alkali. Any appreciable loss of ammonia gives high results, and if the standard alkali is added to the precipitate in an open beaker it is important that the titration be made as soon as possible after the precipitate has dissolved. The rate of loss of ammonia may be decreased by adding about 50 cc. of carbon-dioxide-free water before the addition of the standard alkali. If the precipitate is dissolved in a stoppered Erlenmeyer flask, no loss of ammonia can occur and prompt titration of the excess of alkali is then unnecessary.

(c) The end point may be determined most accurately by adding a little more indicator towards the end of the titration and by comparing the color of the solution with one to which no indicator has been added, or which has already been titrated. As a check on the titration, an excess of acid may be added and the titration continued by the addition of the standard alkali until the reappearance of the pink color.

(2) Withdraw a second 10 cc. aliquot of the solution prepared as directed under A (1), add 0.5 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under A (1).

(3) Withdraw a third 10 cc. aliquot of the solution prepared as directed under A (1), add 10 grams of ammonium nitrate, acidify with a few drops of nitric acid, dilute to 75–100 cc., place in a stirring apparatus, add 50 cc. of the molybdate solution, stir for at least 30 minutes at room temperature, filter, wash, and complete the determination as directed under A (1).

(4) Withdraw a fourth 10 cc. aliquot of the solution prepared as directed under A (1), add 0.5 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under A (3).

B. Determine total P_2O_5 in Sample No. 2 by the following procedures:

(1) Dry the sample at 105°C. for 1 hour. Dissolve 2.5 grams of the sample in 30 cc. of strong nitric acid and 3–5 cc. of strong hydrochloric acid, boil until the organic matter is destroyed, and dilute to 500 cc. with water. Mix and allow to settle, or pour on a dry filter; withdraw an aliquot of 20 cc., add 10 grams of ammonium nitrate and then strong ammonia in slight excess, and barely dissolve the precipitate formed with a few drops of nitric acid (1 + 1), stirring vigorously; dilute to 75–100 cc. and complete the analysis as directed under A (1).

(2) Withdraw a second 20 cc. aliquot of the solution prepared as directed under B (1), add 0.5 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under B (1).

(3) Withdraw a third 20 cc. aliquot of the solution prepared as directed under B (1), add 10 grams of ammonium nitrate and then strong ammonia in slight excess, and barely dissolve the precipitate formed with a few drops of nitric acid (1 + 1), stirring vigorously; dilute to 75–100 cc., place in a stirring apparatus, add 50 cc. of the molybdate solution, and stir for at least 30 minutes at room temperature; filter, wash, and complete the determination as directed under A (1).

(4) Withdraw a fourth 20 cc. aliquot of the solution prepared as directed under B (1), add 0.5 gram of potassium sulfate and 10 grams of ammonium nitrate, and complete the determination as directed under B (3).

ANALYSIS OF STANDARD PHOSPHATE SAMPLES.

The results reported by the collaborators are given in Table 1.

COMMENTS BY ANALYSTS.

W. R. Austin.—The temperature of solution at time of precipitation causes more variations than any other factor, and the expression "room temperature" is therefore

TABLE 1.
Analysis of standard phosphate samples.

COLLABORATORS	SAMPLE NO. 1—52.18 PER CENT P_2O_5				SAMPLE NO. 2—31.33 PER CENT P_2O_5			
	Precipitation with- out sulfates at		Precipitation with sulfates at		Precipitation with- out sulfates at		Precipitation with sulfates at	
	45°-50°C.	Room tempera- ture, with stirring	45°-50°C.	Room tempera- ture, with stirring	45°-50°C.	Room tempera- ture, with stirring	45°-50°C.	Room tempera- ture, with stirring
W. R. Austin Armour Fer- tilizer Works	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
	52.34	52.34	54.14	52.34	31.61	31.40	32.40	31.41
C. M. Bible Mellon Insti- tute Industrial Research	52.38	52.31	53.50	52.38	31.50	31.32	32.10	31.50
R. D. Caldwell Armour Fer- tilizer Works	52.48	51.72	55.40	52.12	31.27	31.34	31.68	31.42
F. O. Lundstrom Bureau of Chemistry and Soils	52.60	52.66	53.08	52.70	31.71	31.53	32.48	31.66
James B. Martin Bureau of Chemistry and Soils	52.43	52.22	53.18	51.98	31.35	31.31	31.97	31.40
P. McG. Shuey Shuey & Co.	52.70	52.18	56.11	53.00	31.90	31.45	33.54	32.17
D. S. Reynolds Bureau of Chemistry and Soils	52.20	52.03	53.71	52.29	31.20	31.10	31.58	31.38
W. A. Ryder F. S. Royster Guano Co.	52.20	52.20	53.80	52.80	31.35	31.30	32.30	31.40
Mean	52.42	52.21	54.11	52.45	31.49	31.34	32.26	31.54

not sufficiently definite. When using a stirring machine I add the molybdate solution at a cool temperature, place in a water bath or add enough warm water to bring to about 35°C. before placing in the machine. Only by this procedure can I obtain consistent results on my check standards winter and summer.

C. M. Bible.—Too great excess of molybdate solution should be avoided. At room temperature the difference due to an excess of 20 cc. amounts to about 0.20 per cent, while at 45°C. the difference is still greater, particularly when sulfates are present. The precipitates made at room temperature were much more easily washed free from acid than those prepared at 45°-50°C.

P. McG. Shuey.—The addition of 0.5 gram of potassium sulfate, as specified in the directions, supplies four times the sulfate radicle usually present in the quantity of superphosphate recommended for analysis, and when the latter is analyzed by precipitation at 30°C. interference of sulfates may be considered negligible. Precipitation at room temperature is by far the best procedure to employ and the only one that should be considered official. The amount of molybdic solution as well as the length of time of stirring has a tendency to influence results, and this influence seems to be greater when sulfates are present or when precipitation is made above room temperature.

I should like to urge the use of sulfuric acid as a solvent in the preparation of organic materials for analysis by the volumetric method. This method of preparing a solution of the sample is being used by some of the State chemists and by many others, and while not official, I think it should be.

DISCUSSION OF RESULTS.

Table 1 shows, in agreement with previous work on the subject, that the presence of sulfates seriously interferes in the volumetric analysis of phosphates when precipitation is made at 45°–50°C., but that the interference is slight when precipitation is made at room temperature with stirring.

The room temperatures at which these results were obtained represent summer temperatures and were therefore more uniform than if the determinations had been made at different seasons throughout the year. It is well known that the rate of precipitation and the composition of the phosphomolybdate precipitate varies with the temperature of the solution to which the molybdate is added. Austin's contention that "room temperature" is not sufficiently definite would thus appear to be well taken; therefore the step of bringing the solution to a temperature of about 30°C. before the addition of the precipitating agent should be adopted when necessary.

It would seem that when a suitable stirring or shaking machine is available the procedure of precipitating at an average room temperature and stirring for 30 minutes requires less attention than that of precipitating at 45°–50°C. and maintaining this temperature for 30 minutes with occasional stirring. The greater convenience of the former procedure and its greater accuracy in the analysis of commercial phosphates would seem to warrant its adoption in place of the alternative official method of precipitating at 45°–50°C. No tests were made, however, on the analysis of materials high in organic matter, and it is considered that this work is necessary before any definite action is taken.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the work be continued next year on a study of the volumetric analysis of phosphates in the presence of sulfates and organic matter.
- (2) That further study be made of the effect of sulfates on the volu-

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 69.

metric method for phosphoric acid, smaller quantities of sulfates being used with a view to deleting the precipitation at 45°–50°C.

The following recommendations on which first action was taken in 1927 are again presented for final action:

(1) That the words "*nearly neutralize with strong hydrochloric acid*" in the gravimetric determination of phosphoric acid (p. 3, 7, line 12) be changed to read: "*neutralize with strong hydrochloric acid, using litmus paper or bromthymol blue as indicator*".

(2) That the words "*burn first at a low heat and then ignite intensely until white or grayish white*" (p. 3, 7, line 17), in the gravimetric determination of phosphoric acid be changed to read: "*burn first at a low heat and ignite to constant weight, preferably in an electric furnace at 950°–1000°C.*".

(3) That a third alternative method for the preparation of magnesia mixture (p. 2, 5 (c)) be worded as follows:

(3) Dissolve 55 grams of crystallized magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in water, add 140 grams of ammonium chloride, and dilute to 870 cc. Add strong ammonium hydroxide to each required portion of the solution just before using at the rate of 15 cc. per 100 cc. of solution.

REPORT ON NITROGEN.

By A. L. PRINCE (Agricultural Experiment Station¹, New Brunswick, N. J.), Associate Referee.

The methods in use at the present time for distinguishing sharply between organic and mineral nitrogen when cyanamide, urea, and acid fish are used in fertilizer mixtures are not entirely satisfactory. The Arnd², Devarda³, and reduced iron methods⁴ are known to give erroneous results in the presence of such materials. While the Jones method⁵ is a distinct improvement over the above-mentioned methods, it is not so accurate and rapid as desired.

It was suggested last year that the Associate Referee on Nitrogen try out a method for the determination of mineral nitrogen in commercial fertilizers in the presence of calcium cyanamide and urea, proposed by B. F. Robertson, Clemson Agricultural College. This method has not been published. It was also recommended that this method be compared with the Jones method as to accuracy and speed. An endeavor to carry out this recommendation in the time that could be devoted to the work was made. Extensive collaborative assistance was

¹ Journal Series Paper of the New Jersey Agricultural Station, Department of Soil Chemistry and Bacteriology.

² Z. angew. chem., Aufsatzteil 1, 1917, 30: 169.

³ Methods of Analysis, A. O. A. C., 1925, 12.

⁴ Ibid., 11.

⁵ Ind. Eng. Chem., 1927, 19: 269; This Journal, 1928, 11: 32.

not considered necessary before it had been ascertained whether the method was worth further study, but judging from the preliminary work, it would appear that it would be fully warranted another year.

The principle involved in the proposed Robertson method may be briefly stated as follows: An aliquot of the fertilizer solution is boiled with sulfuric acid and ferrous sulfate. In the reduction of the nitrates, the nitrogen monoxide (NO) produced combines with the ferrous sulfate to form an unstable compound— $7\text{FeSO}_4 + 3\text{H}_2\text{SO}_4 + 2\text{HNO}_3 (\text{NO}_2) + 3\text{Fe}_2 (\text{SO}_4)_3 + 4\text{H}_2\text{O}$. Thus, the nitrate nitrogen is driven off. The water-soluble nitrogen is previously determined by subtracting the insoluble nitrogen from the total nitrogen. Then the difference between the water-soluble nitrogen and the nitrogen obtained after the ferrous sulfate digestion gives theoretically the nitrate nitrogen. The procedure as carried out subsequently is as follows:

1. Determine the total nitrogen by the usual method.
2. Weigh out 2 grams of the fertilizer mixture, wash to a volume of 200 cc., and determine the nitrogen in the residue by any of the modifications of the Kjeldahl method. The difference between these two determinations gives the water-soluble nitrogen.
3. Distil 50 cc. of the filtrate, equivalent to 0.5 gram, with magnesium oxide for ammoniacal nitrogen as described in *Methods of Analysis*, p. 11.
4. Put another 50 cc. portion of the same solution into a 500 cc. Kjeldahl flask, together with 2 grams of ferrous sulfate and 20 cc. of sulfuric acid, sp. gr. 1.84. Digest over a hot flame. After the water is evaporated and white fumes appear, continue the digestion for at least 10 minutes. The nitrate nitrogen is thereby driven off. (If the solution does not clear, add 0.65 gram of mercury and boil until clear, then after dilution add potassium sulfide solution to precipitate mercury as usual.) Cool, dilute, and distil with strong caustic soda in the usual way. Add pinch of a mixture of zinc dust and granular zinc (20 mesh) to each flask before distillation to prevent bumping.

One (total) minus two (water-insoluble) gives water-soluble nitrogen. The difference between the water-soluble nitrogen and the nitrogen obtained in Part 4 of the directions gives the nitrate nitrogen. Three (ammonia nitrogen) plus nitrate nitrogen gives total mineral nitrogen. Total nitrogen less mineral nitrogen gives the organic nitrogen.

The Robertson method was run on several samples that had been prepared the previous year for work on the Jones method. The results obtained in comparison with those obtained by the Jones and reduced iron methods are included in Table 3. Two new samples, No. 5 and No. 6, were prepared as follows:

	PARTS PER 100		NITROGEN— BY ANALYSIS	NITROGEN IN SAMPLE— CALCULATED	
	No. 5	No. 6		No. 5	No. 6
			per cent	per cent	per cent
Superphosphate.....	60	60			
Potassium chloride....	10	10			
Sodium nitrate.....	10	10	15 90	1 59	1 59
Calcium cyanamide.....	5	5	19 93	1.00	1.00
Urea.....	5		46 39	2.32	
Ammonium sulfate.....	5	5	20 81	1 04	1.04
Dried fish.....	5	10	7 91	0.40	0.79
	100	100		6 35	4 42

The cyanamide and urea used in sample No. 5 were equivalent to 100 pounds per ton of each substance; 100 pounds per ton of cyanamide was also used in sample No. 6.

The results of the analyses of these two samples by the associate referee and by Robertson are given in Table 1. The quantities of the various forms of nitrogen obtained by three different methods are reported. The calculated nitrate nitrogen content of these two samples was 1.59 per cent. In the analyses of these two samples made by the associate referee, the nitrate nitrogen content by the Robertson method was 1.67 and 1.62 per cent; by the Jones method, 1.75 and 1.80 per cent; and by the reduced iron method, 2.18 and 2.20, respectively. Obviously, the reduced iron method gave results too high for nitrate nitrogen, with a corresponding low value for the organic nitrogen. The results with the Jones method were a decided improvement over those with the reduced iron method, but they were also rather high for nitrate nitrogen. The figures obtained with the Robertson method are nearer the calculated values. The results of Robertson's analyses of these two samples may be seen in the adjoining columns of the table; they follow the same general trend as those of the associate referee. The Jones method appears to yield slightly higher results than the Robertson method for nitrate nitrogen. Robertson also ran the Arnd alloy method for mineral nitrogen in place of the reduced iron method. It appears from the results he obtained that the Arnd method is superior to the reduced iron method, but the Arnd method also gives too high results for mineral nitrogen and a low value for organic nitrogen.

The samples were also sent to J. E. Breckenridge at Carteret, N. J., for further collaborative study. A comparison of his results in condensed form with those already mentioned may be seen in Table 2. In general, the work reported by these collaborators substantiates the results obtained by the associate referee. Breckenridge made the following comment in connection with his results: "This matter of mineral and organic nitrogen seems to be the most unsatisfactory test that we have. It is hard to secure closely agreeing results on mineral nitrogen according to the reduced iron method when this method is carried out by different analysts, even in the same laboratory".

Another point brought out in the study of this problem is that the extent of error in running the reduced iron method is proportional to the quantity of cyanamide or urea present in the fertilizer. This fact can be clearly seen from Table 3. In column 1 are shown the results of analyzing a fertilizer containing only 41.3 pounds of cyanamide per ton; in columns 2 and 3 results of analyzing a fertilizer containing 100 pounds and 250 pounds of cyanamide per ton are given. With only 41 pounds of cyanamide, the error by the reduced iron method is not great, being only 0.07 per cent above the calculated value. With 100 pounds of

TABLE 1.

Analysis of samples No. 5 and No. 6.
(Results expressed in percentage of nitrogen.)

DETERMINATIONS	CALCULATED VALUES		ROBERTSON METHOD				JONES METHOD				REDUCED IRON METHOD			
	Sample—		Associate reference		Robertson		Associate reference		Robertson		Associate reference		Robertson	
			Sample—		Sample—		Sample—		Sample—		Sample—		Sample—	
	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6	No. 5	No. 6
Total nitrogen	6.35	4.42	0.43	0.78	6.21	4.38					6.43	4.51		
Water-insoluble nitrogen			6.00	3.73	5.80	3.69								
Water-soluble nitrogen			4.33	2.11	4.17	2.12								
Nitrogen after ferrous sulfate treatment			1.67	1.62	1.63	1.57								
Nitrate nitrogen	1.59	1.59	1.12	1.05	0.95	0.89	1.75	1.80	1.70	1.74	2.18	2.20	2.81	2.80
Ammonia nitrogen	1.04	1.04	2.79	2.67	2.58	2.46	1.13	1.10	0.95	0.89	1.12	1.09	Arnd method	Arnd method
Mineral nitrogen	2.63	2.63					2.88	2.90	2.65	2.63	3.30	3.29	3.40	1.58
Organic nitrogen	3.72	1.79	3.64	1.84	3.64	1.92	3.55	1.61	3.57	1.74	3.13	1.22	Arnd method	Arnd method

cyanamide the error is 0.6 per cent above the calculated value, and with 250 pounds the error increases to 1.23 per cent. However, when the Robertson method is used on these samples, the deviation from the calculated value is never greater than 0.04 per cent, while the deviation with the Jones method is between 0.2 and 0.3 per cent. Seventeen pounds of urea per ton caused no error in the analyses for nitrate nitrogen by the reduced iron method, as is shown in column 5, but 250 pounds per ton caused an error of 0.43 per cent. Either the Robertson or Jones method brought the error down to about 0.05 per cent.

From this preliminary study, it is the opinion of the associate referee that the Robertson method is slightly more accurate and also simpler than the Jones method. It would seem advisable, however, to make a more extensive collaborative study of these two methods before recommending the adoption of the Robertson method to replace the Jones method.

Two other recommendations, both of which received first action last year, are included.

RECOMMENDATIONS¹.

It is recommended—

(1) That further collaborative work be done on comparing the Robertson and Jones methods for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea.

(2) That the official zinc iron method for the determination of nitric and ammoniacal nitrogen be discarded (final action).

(3) That the official reduced iron method for the determination of nitric and ammoniacal nitrogen be marked "Applicable only in the absence of cyanamide and urea" (final action).

TABLE 2.

Averages of collaborative results.
(Expressed as percentage of nitrogen.)

ANALYST	NITRATE NITROGEN CALCULATED VALUE 1.59 PER CENT					
	Robertson method		Jones method		Reduced iron method	
	Sample 5	Sample 6	Sample 5	Sample 6	Sample 5	Sample 6
Associate Referee.....	1.67	1.62	1.75	1.80	2.18	2.20
Breckenridge.....	1.56	1.64	1.64	1.73	1.97
Robertson.....	1.63	1.57	1.70	1.74

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 70.

TABLE 3.
Results of determination of nitrate nitrogen in mixed fertilizers containing various quantities of cyanamide and urea.

METHOD USED	41.3 LBS. CYANAMIDE PER TON		100 LBS. CYANAMIDE PER TON		250 LBS. CYANAMIDE PER TON		MIXTURE OF 100 LBS. CYANAMIDE AND 100 LBS. UREA PER TON		17.7 LBS. UREA PER TON		250 LBS. UREA PER TON	
	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found
Reduced Iron Method	1.65	1.72	1.59	2.20	1.94	3.17	1.59	2.18	1.65	1.66	1.94	2.37
Robertson Method	1.65	1.61	1.59	1.62	1.94	1.96	1.59	1.67	1.65	1.60	1.94	2.02
Jones Method	1.65	1.62	1.59	1.80	1.94	2.22	1.59	1.75	1.65	1.63	1.94	1.99

REPORT ON NITROGEN ACTIVITY METHODS IN FERTILIZERS.

DETERMINATION OF ACTIVE WATER-INSOLUBLE NITROGEN BY THE ALKALINE PERMANGANATE METHOD.

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.),
Associate Referee.

In 1926 Moore and White¹ directed attention to the need for more definite directions for the technic of the alkaline-permanganate method for the activity of insoluble-nitrogen². Magruder³ and others have noted a lack of uniformity in the results reported by different laboratories. Investigational work, reported in 1927⁴, resulted in recommendations for changes in the method, many of which had been a part of the method previously, but had been omitted from subsequent writings. Collaborative work was undertaken in 1928 to see if the changes had brought improvement in uniformity.

SAMPLES.

Three samples were distributed. Sample No. 1 was an organic material furnished by the Sewerage Commission of the City of Milwaukee, and sold by them under the trade name "Milorganite". Samples No. 2 and 3 were from a 4-12-4 mixed fertilizer prepared by Robert White of the Armour Fertilizer Works. The insoluble nitrogen was supplied by a 10 per cent nitrogenous tankage; the remainder of the nitrogen was supplied by cyanamide, nitrate of soda, and sulfate of ammonia. One part of the mixture was ground to pass a 1 mm. sieve and thoroughly mixed, and subdivisions were distributed as Sample No. 2. Another part was passed through a 10-mesh sieve and mixed, and the subdivisions were labeled Sample No. 3. These portions of Sample No. 3 were ground to pass a 1-mm. sieve at the laboratories of the collaborators. After completion of the analyses, Sample No. 3 was returned to the associate referee for a mechanical analysis.

To demonstrate the uniformity of the subsamples distributed, 5 of each group of 20 were selected at random and analyzed by the associate referee. The analyses are reported in Table 1.

INSTRUCTIONS TO COLLABORATORS.

1. *Preparation of material.* Grind sample No. 3 in accordance with the customary procedure in your laboratory and use the ground sample for analysis. After completion of the work, return the remainder of this ground sample to the associate referee for a mechanical analysis. Mix samples carefully. Determine moisture in the three samples by drying 2 grams for 5 hours at 100°C.

¹ *This Journal*, 1927, 10: 202.

² *Methods of Analysis*, A. O. A. C., 1925, 12.

³ *This Journal*, 1922, 5: 454.

⁴ *Ibid.*, 1928, 11: 191.

TABLE 1.

Analysis of 5 subsamples from each group of 20 prepared for distribution to collaborators.

SUB-SAMPLE	INSOLUBLE NITROGEN	ACTIVITY OF INSOLUBLE NITROGEN	SUB-SAMPLE	INSOLUBLE NITROGEN	ACTIVITY OF INSOLUBLE NITROGEN	SUB-SAMPLE	INSOLUBLE NITROGEN	ACTIVITY OF INSOLUBLE NITROGEN
	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
1 a	5.07		2 a	1.09		3 a	1.04	59.3
1 b	5.08		2 b	1.09		3 b	1.03	61.0
1 c	5.03	60.1	2 c	1.03	59.5	3 c	1.05	60.3
1 d	4.98	58.4	2 d	1.03	59.8	3 d	1.07	
1 e	5.02	60.8	2 e	1.05	58.1	3 e	1.02	

2. *Alkaline permanganate solution.* Dissolve 25 grams of C. P. potassium permanganate in hot water, and, separately, 150 grams of C. P. sodium hydroxide in water; combine the solutions when cold and dilute to 1 liter. Determine the concentration of potassium permanganate in the alkaline solution as follows: Dry the sodium oxalate at 100°C. and weigh 0.5 gram of the dry salt into a 400 cc. beaker. Dissolve in 300 cc. of water and 10 cc. of concentrated sulfuric acid (sp. gr. 1.84). Heat to the boiling point and titrate while hot with the alkaline permanganate solution from an all-glass buret. Titrate to a persistent pink, allowing the buret to drain for 5 minutes before all readings. The long drainage is necessitated by the adhesion of the solution to the walls of the buret. Report concentration of the permanganate (0.2358 divided by titration in cc. gives grams of potassium permanganate per cc.), but do not adjust the solution. Discard solutions more than 14 days old, or repeat the titration and report changes noted.

3. *Water-insoluble nitrogen.* Place 1 gram of the material on an 11 cm. filter paper and wash with water at room temperature until the filtrate measures 250 cc. Dry and determine nitrogen in the residue and paper as directed under I, 19 or 22, *Methods of Analysis*, A. O. A. C., 1925. Fold three similar clean filter papers together, wash, and determine water-insoluble nitrogen found, correcting for nitrogen in the paper.

4. *Preparation of sample.* Prepare duplicate portions, A and B, as follows: Place a quantity of material equivalent to 50 mg. of water-insoluble nitrogen as determined above, on an 11 cm. filter. Wash with water at room temperature until the filtrate measures 250 cc. When it is found necessary to use 4 or more grams of the original material, weigh the required quantity into a small beaker, wash by decantation, finally transfer to the filter, and finish the extraction as previously directed. Dry the residues at a temperature not exceeding 80°C. and transfer A and B from the filter to 500-600 cc. Kjeldahl distillation flasks, loosening adhering particles with a stiff brush. Avoid transfer of brush bristles or of excessive paper fiber. In portion A, determine nitrogen as in Section 3 of these directions. Report the milligrams of nitrogen transferred.

5. *Determination.* To portion B, transferred to a Kjeldahl flask, add 20 cc. of water, approximately 5 cc. of perforated glass beads, a drop of mineral lubricating oil weighing from 20 to 50 mg., and 100 cc. of alkaline permanganate solution. Connect with an upright condenser to the lower end of which has been attached a 100 cc. graduated cylinder containing standard acid, and arranged to receive the distillate below the surface of the acid or otherwise adequately trapped to prevent loss of ammonia fumes. Digest slowly with a very low flame for 30 minutes, barely below distillation point;

using coarse wire gauze with asbestos center between the flask and flame. Gradually raise the temperature and distil exactly 95 cc. in 60 minutes (plus or minus 5 minutes), controlling the distillate so that approximately 24 cc. is obtained in each 15 minute period. Record cc. of distillate after 15, 30, 45, and 60 minutes. Conduct the first part of the distillation over a bare flame, but use wire gauze 10 minutes before completion to avoid breaking the flask. Transfer to an Erlenmeyer flask or a beaker and titrate with standard alkali, using cochineal or methyl red indicator. During the digestion and distillation gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides. Calculate the nitrogen thus obtained and report as percentage of the 50 mg. sample. This is the active water-insoluble nitrogen. Rinse the condenser tubes after each distillation. (This may be done by allowing water to be drawn through the tubes by the suction developed by the cooling Kjeldahl flask.)

COLLABORATORS.

The following collaborators assisted in this work:

1. C. R. Engle and G. J. Kuhlman, Bureau of Foods and Chemistry, Department of Agriculture, Harrisburg, Pa.
2. J. E. Rice, Armour Fertilizer Works, Carteret, N. J.
3. Owen L. Nolan, Agricultural Experiment Station, New Haven, Conn.
4. W. R. Austin, Armour Fertilizer Works (Tennessee Chemical Co.), Nashville, Tenn.
5. H. L. Moxon, Virginia Carolina Chemical Corporation, Richmond, Va.
6. John B. Smith, Rhode Island Agricultural Experiment Station, Kingston, R. I.
7. M. W. Goodwin, Massachusetts Agricultural Experiment Station, Amherst, Mass.
8. Percy O'Meara, Michigan Department of Agriculture, Lansing, Mich.
9. Bradley W. Bangs, American Agricultural Chemical Co., Carteret, N. J.
10. B. L. Samuel, Department of Agriculture of Virginia, Richmond, Va.

DISCUSSION.

The collaborative results are reported in Tables 2, 3, and 4. In most instances, three results were submitted by the analysts for each determination. The exceptions were the analyses for moisture and for the concentration of potassium permanganate in the alkaline solution. Only the averages of the results from each analyst have been compiled in the tables.

The results for insoluble nitrogen are in excellent accord. Significant deviations appear, however, in securing 50 mg. of insoluble nitrogen in the Kjeldahl flask for treatment with the alkaline permanganate solution. Since the deviations are greater than the error in determining

nitrogen, they must be caused either by failure of the technic to leave 50 mg. of nitrogen in the residue on the filter after washing the proper quantity of sample with 250 cc. of water, or by the impossibility of transferring all the residue to the flask. Of the 30 results, 17 show between 49 and 51 mg. transferred. For a nitrogenous material showing an activity of 60 the above limits would allow for a maximum error of only 1 unit, but the largest possible difference between two results would be twice that value. Until a better technic can be devised, it is advisable to correct results for this error by calculating the activity on the basis of the average quantity of nitrogen placed in the flask, as shown by analyses of duplicate portions of material treated similarly to that intended for distillation with the permanganate solution. This correction, made for the data reported in Table 3, tended to increase the activities slightly and, in most cases, undoubtedly gave a value nearer to the truth. An interesting example is the low activity found by Collaborator 1 for Sample No. 3. This was caused by an apparently high result for insoluble nitrogen that was responsible for a shortage of 6 mg. of nitrogen in the residue used subsequently for the distillation. The correction brought the data into good accord with the mean. This is also true for the data for Sample No. 2, Collaborator 4. An exception may be noted for the data from Collaborator 10. In this case high values for activity were increased by the correction.

The care that was exercised in governing the rate of distillation is evident from data reported in Table 4.

Although the data are too few to afford reliable constants, the probable errors for single results were calculated and the values are stated in Tables 2 and 3. The formulae used were:

$$\text{Standard deviation, } \sigma = \sqrt{\frac{\sum d^2}{N-1}} .$$

Probable error of a single determination, $Es = 0.6475 \times \sigma$. Wood and Stratton¹ state, "The probable error thus determined is a measure of the reliability of any one result. It is such that taking any single result at random, the chances are even for or against that result differing from the average by the amount of the probable error". The probable error will be the same, whether calculated from all the results submitted or from the averages of the results of each analyst. It should not be confused with the probable error of the mean.

If the ratios of the error to the mean are expressed as percentages, it may be seen that the errors for three important steps in the technic are of the same general magnitude, i. e., all are less than 5 per cent. This might be interpreted as placing the chief responsibility for error on

¹ *J. Agr. Sci., England*, 1910, 3: 417.

the first step, the determination of the insoluble nitrogen. An attempt at correction for this error by calculating the activity from the nitrogen actually treated, does not, however, decrease the error of the final result.

No great confidence should be placed in constants calculated from so few results and from only two types of material, but it is interesting to observe that the chances are even that a determination for activity will fall within plus or minus 3 per cent of the mean of a number of determinations, equivalent to 2 units of activity for a 60 per cent material. If the highest result and the lowest result are eliminated from the data in Table 3, the probable error is reduced by one-half.

EFFECT OF VARIATIONS IN GRINDING.

No significant differences appear to have been caused by individual variations in grinding Sample No. 3, nor do the mechanical analyses, all made by one analyst, show many such differences (Table 5). It was found necessary to dry the material and to break up the lumps of superphosphate (acid phosphate) before consistent results could be secured. The color of the siftings indicated that little of the nitrogenous tankage passed a 200-mesh sieve.

EFFECT OF CONCENTRATION OF THE POTASSIUM PERMANGANATE.

There is a considerable degree of correlation between the concentrations of potassium permanganate and the corrected values for activity. If the latter are divided into groups in accordance with increasing permanganate concentrations, the average activities of the groups increase regularly (Table 6). Better uniformity is possible, and titration of the solution with an oxalate, followed by adjustment, is advised. It is probable that stock solutions of the permanganate could be standardized and kept for some time if unmixed with the sodium hydroxide. Fresh solutions could be prepared for immediate use by combining the stock solution containing the permanganate with a second solution containing the hydroxide.

COMMENTS BY COLLABORATORS.

O. L. Nolan found between 1 and 2 mg. of nitrogen left on and in the filter paper after transfer of the residue to the Kjeldahl flask.

W. R. Austin wrote:

We also carried these along by a procedure which included ether washing of the samples, use of rock dust, also a variation in digestion which we have been using here for several years. This consists of digestion in the cold solution for 30 minutes, then raising to the boiling point during the next 30 minutes; after this the distillation period was 1½ hours to get 95 cc.

The results obtained were uniformly higher by three units of activity than those reported for the collaborative procedure.

H. L. Moxon commented as follows:

A good grade of filter paper should be used as different grades give varying results. The paper used in making these analyses was B & A, Grade A. It has been found impossible to distil the exact amount each 15 minutes, but the 95 cc. can be collected in about the right time.

The comment concerning filter paper is significant in view of Moxon's success in securing 50 mg. samples for treatment with the permanganate solution.

Percy O'Meara commented as follows:

The amount of insoluble nitrogen actually transferred seems to vary almost as much, and affects the final calculated percentage nearly as much, as the variation in ammonia distilled. Both seem to be critical points in the method as well as the rate of distillation.

If there is an allowable variation in size of Kjeldahl flask for the permanganate distillation it ought to be determined, since several laboratories use 800 cc. flasks altogether. In this work I used the only 500 cc. flask I had for half the distillations, and an 800 cc. flask for the others. I do not notice any particular trend in those results, although the small flask seems to require less attention and care during the distillation.

I used the gas flame and asbestos gauze as specified for all the work, but hope to get time later to check it against our regular electric installation of Gilmer heaters which fit the 800 cc. flasks which we use in all our routine nitrogen work. If such apparatus is not suitable for activity determinations, we certainly want to know it, and, on the other hand, if it can be used, it would seem a good thing if the method could be worded to permit it. I had great difficulty in keeping the gas flame adjusted for the rate of distillation specified, especially if there were any open windows in the room. I realize, of course, a like difficulty in making electric heaters distil at that rate, since, as ordinarily used, they distil at about twice the rate called for in this determination. But it may be possible for the individual analyst to work out a combination of asbestos discs and distance from the heating coils to obtain the required rate, and then it would be more independent of drafts than a gas flame.

H. D. Haskins and M. W. Goodwin commented as follows:

With reference to the water-insoluble nitrogen transferred to the Kjeldahl flask, it has been our custom to make this transfer in the wet condition. We take a circular piece of tin and bend it so that it forms a smooth trough. The wet filter paper is carefully unfolded and placed in this, and we use a pipet drawn out so that it discharges a very fine stream; 20 cc. of water is then used to wash the residue from the wet filter paper after poking most of it into the flask by means of a glass rod or a platinum spatula. Determinations run on this method of transfer give the following results:

Water-insoluble nitrogen transferred to Kjeldahl flask.

SAMPLE	1	2	3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
a	49.90	48.60	48.60
b	49.80	48.50	48.80
c	49.80	48.20	49.10

You will note from this method of transfer that considerably more insoluble nitrogen was transferred to the flask than is the case with the method of drying the residue before transferring to the flask. I believe that this is worth adopting by chemists making this activity test.

This procedure was allowed by the method previously and is responsible for the addition of 20 cc. of water to the material in the flask after transfer in a dry state. It has the advantage of allowing immediate use of a wet residue and eliminates paper fibers or brush bristles from the transferred material.

The associate referee was unable to confirm the evidence noted by Goodwin, that a larger quantity of insoluble nitrogen may be transferred by the wet method than by the dry. For three samples, the average numbers of milligrams for two or more trials by each of the procedures were 49.1 vs. 48.2, 48.0 vs. 48.0, 47.8 vs. 47.9. The methods seem equally good, and the analyst should be allowed a choice if further study confirms the above findings. The possible effects of drying the protein material of the sample should also be considered.

CONCLUSIONS.

Sufficient study of the method has been made to indicate a considerable degree of probability that the results from a single laboratory will not differ from the mean for several laboratories by more than 3 per cent of that mean. This is not the ultimate limit of accuracy for the method, but it should be regarded as encouragement to continue the study of details that allow deviations in technic.

TABLE 2.

Collaborative results for moisture, insoluble nitrogen, and quantity of nitrogen transferred to Kjeldahl flask from a washed sample calculated to contain 50 mg. of insoluble nitrogen.

COLLABORATOR	MOISTURE			INSOLUBLE NITROGEN*			INSOLUBLE NITROGEN TRANSFERRED*		
	Sample			Sample			Sample		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	5.60	5.19	5.23	4.90	1.02	1.14	50.2	49.9	44.1
2	5.00	5.70	5.50	4.89	1.00	0.99	50.1	50.0	49.8
3	5.12	5.82	4.98	1.00	1.01	49.4	49.1	48.7
4	5.30	5.90	7.00	5.10	1.09	1.00	49.6	45.7	48.3
5	4.78	4.58	5.05	5.03	1.02	0.99	50.1	50.0	50.0
6	7.34	7.31	7.10	5.03	1.03	1.03	48.2	48.0	48.2
7	5.08	4.63	4.58	4.97	1.08	1.07	49.7	46.5	47.3
8	5.56	5.53	6.17	4.90	1.00	1.02	49.7	50.2	48.4
9	10.48	5.08	6.58	4.94	1.00	0.95	51.2	52.0	50.6
10	4.12	4.84	5.64	5.01	0.99	1.00	47.9	47.9	47.6
Mean	4.98	1.02	1.02	49.6	48.9	48.3
E _s †	±0.05	±0.02	±0.05	±0.7	±1.3	±1.8
E _s ×100	1%	2%	4.9%	1.4%	2.6%	3.7%

* Averages of 3 results usually reported.

† Probable error of a single result.

TABLE 3.

Collaborative results for the activity of insoluble nitrogen.

COLLABORATOR	ALKALINE PERMANGANATE SOLUTION (KMnO ₄)		ACTIVITY OF INSOLUBLE NITROGEN*					
			Basis of 50 mg. nitrogen assumed to be in flask			Basis of mg. of nitrogen found in flask by analysis		
			Sample			Sample		
			No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
	<i>days</i>	<i>grams per L.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	13	21.2	54	59	54	54	59	61
2	6	22.2	58	60	60	58	60	60
3	6	22.5	59	62	60	60	63	62
4	7	23.6	60	58	61	61	63	63
5	1	24.8	60	62	62	60	62	62
6	1	24.3	60	60	61	62	63	63
7	2	24.3	61	62	62	61	67	66
8	1	22.5	62	60	63	62	60	65
9	1	23.1	63	62	63	62	60	62
10	2-7	24.0	64	65	66	67	68	69
Mean	60	61	61	61	63	63
E _s †	±2	±1	±2	±2	±2	±2
E _s × 100	3.3%	1.6%	3.3%	3.3%	3.2%	3.2%
m								

Highest result and lowest result omitted.

Mean	60	61	61	61	62	63
E _s †	±1	±1	±1	±1	±2	±1
E _s × 100	1.7%	1.6%	1.6%	1.6%	3.2%	1.6%
m								

* Averages of results submitted, usually 3.

† Probable error of a single result.

RECOMMENDATIONS¹.

It is recommended—

(1) That the study to secure greater uniformity in results for the activity of water-insoluble nitrogen by the alkaline-permanganate method be continued and that the following points be studied intensively:

a. The advantages of a 5 gram sample for diminishing the error in the determination of water-insoluble nitrogen.

b. The preparation of a standard alkaline-permanganate solution from stock solutions of potassium permanganate and of sodium hydroxide that have been standardized by titration.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 70.

TABLE 4.

Averages of collaborative results showing rate of distillation with alkaline-permanganate solution.

Collaborator.....	1	2	3	4	5	6	7	8	9	10
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Sample 1										
After 15 min.....	20	21	25	25	33	21	23	28	23	26
After 30 min.....	66	50	52	49	55	45	48	50	50	50
After 45 min.....	92	76	76	73	73	70	73	73	76	71
Sample 2										
After 15 min.....	20	24	24	24	32	21	23	23	25	25
After 30 min.....	54	52	51	49	48	46	49	51	47	51
After 45 min.....	72	72	74	72	67	71	75	72	74	73
Sample 3										
After 15 min.....	25	25	27	25	33	20	23	22	23	24
After 30 min.....	56	48	51	49	51	48	48	52	51	49
After 45 min.....	77	72	70	73	69	74	75	70	74	72
Average										
After 15 min.....	22	23	25	25	33	21	23	24	24	25
After 30 min.....	59	50	51	49	51	46	48	51	49	50
After 45 min.....	80	73	73	73	70	72	74	72	75	72

TABLE 5.

Results of mechanical analysis of sample No. 3 as ground at different laboratories.

(Expressed as percentage of oven-dried material.)*

Collaborator.....	1	2	3	4	5	7	8	9	10
Mesh									
40 ..	4	4	5	4	7	4	5	5	7
40-100 ..	8	8	8	8	8	9	9	18	10
100-170 ..	6	5	6	4	5	4	6	5	4
170-200 ..	3	3	2	4	3	4	2	1	3

* The samples were dried at 105°C., rubbed in a mortar with a rubber pestle to pulverize lumps, sifted, and redried before weighing.

TABLE 6.

Effect of concentration of potassium-permanganate.

KMnO ₄ GRAM PER L.	NITROGEN ACTIVITY*			GROUP AVERAGE		
	Sample			Sample		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
	per cent	per cent	per cent	per cent	per cent	per cent
21-22	54	59	61	54	59	61
22-23	58	60	60			
	62	60	65	62	61	62
	60	63	62			
23-24	62	60	62			
	61	63	63	62	62	63
24-25	67	68	69			
	62	63	63			
	61	67	66			
	60	62	62	63	65	65

* Corrected for quantity of nitrogen entering the reaction.

c. The effects of transferring the washed residue to the flask while the material is wet, as compared with the present method, with a view to the ultimate inclusion of the two processes as alternatives.

(2) That the application of the method to mixtures containing uric acid be studied.

(3) That the changes accepted last year (first reading) be adopted finally.

REPORT ON POTASH.

By L. D. HAIGH (Agricultural Experiment Station, Columbia, Mo.),
Associate Referee.

Last year Subcommittee A recommended (1) that further study of the use of calcium carbonate be made in connection with the official method for potash; and (2) that the Weems method for the direct titration of chlorine in the water-soluble portion of mixed fertilizers be adopted as tentative.

The associate referee was responsible for the suggestion that calcium carbonate be used in the preliminary washing specified in the official method for the determination of potash in mixed fertilizers. In an article last year¹ he offered the explanation that the use of calcium carbonate may be unnecessary provided platinum dishes are used. Therefore, owing to lack of time it was thought best to study only the tentative method for chlorine as adopted last year.

DIRECT TITRATION (WEEMS) METHOD FOR CHLORINE IN MIXED FERTILIZERS.

Two mixtures were prepared for study; they differed from the mixtures used last year in the use of dried blood in place of cyanamide and cotton-seed meal as the organic nitrogen carrier. The mixtures and their constituents were as follows:

Mixture No. 1.—Acid phosphate, dried blood, ammonium sulfate, potassium chloride.

Mixture No. 2.—Acid phosphate, dried blood, sodium nitrate, potassium chloride.

The acidity of the washings from mixture No. 1 was determined by titration, and sufficient dilute ammonium hydroxide of known strength was added to the dry mixture to make it neutral or slightly ammoniacal. The mass was then dried on the water bath, reground, and remixed. The resulting product showed a pH of 5.9–6.0, indicating only a partial success in making this mixture neutral. Mixture No. 2 gave washings showing a pH of 4.8.

¹ *This Journal*, 1928, 11: 219.

The report on potash last year considered only the chlorine added as potassium chloride as the theoretical amount present. This year the percentage of chlorine in each constituent used in the mixture was determined by the Weems method, and the theoretical percentage of chlorine in the finished mixture was figured.

It was thought that the acidity of the washings would influence the result for chlorine when titrated directly according to the method studied. Therefore, as a modification of the direct titration method, a small quantity of calcium carbonate was added to the portion to be titrated, to overcome the acidity present before the addition of the standard silver nitrate solution.

Directions supplied to the collaborators were as follows:

DIRECTIONS FOR TITRATION OF CHLORINE IN FERTILIZERS.

REAGENTS.

(2) *Standard silver nitrate solution.*—Dissolve 5.218 grams of pure recrystallized silver nitrate in distilled water and make up to 1000 cc. volume. Standardize against pure dry sodium chloride. 1 cc. = 0.001 gram of chlorine.

(2) *Potassium chromate solution.*—Dissolve 5 grams of potassium chromate in 100 cc. of distilled water.

(3) *Precipitated calcium carbonate.*—Free from chlorides.

PROCEDURE.

Weigh out in duplicate 2.5 grams of each of the mixtures furnished (No. 1 and No. 2). Place each portion on an 11 cm. filter (which has been washed two or three times with distilled water) and wash the sample with successive portions of boiling distilled water, receiving the filtrate in a 250 cc. flask. Cool to room temperature, make up to mark and pipet from each flask four 50 cc. aliquots into as many 250 cc. beakers. To each beaker add 1 cc. of potassium chromate solution, and to two of the beakers add also 0.5 gram of precipitated calcium carbonate free from chlorides. Titrate all four of the mixtures with standard silver nitrate solution until a permanent red color of silver chromate is formed. Report the results as percentage of chlorine, on each aliquot taken.

The results obtained by the collaborators are shown in the table.

DISCUSSION OF RESULTS.

Two difficulties might be anticipated in attempting to titrate the washings from a mixed fertilizer directly with silver nitrate: (1) the acidity of the solution, which would delay the appearance of the silver chromate marking the end point; and (2) the presence of ammonium salts which form combinations with silver salts. The results reported by the collaborators, with and without the use of calcium carbonate, do not indicate any distinct difference due to acidity. In regard to effects from ammonium salts, it was observed that both mixtures show varying results, all less than the theoretical quantity of chlorine present. Mixture No. 1 contained ammonium salts, but mixture No. 2 did not.

There is evidently no consistent difference in these results which would point to the effect of ammonium salts on the titration.

All the results obtained were higher than the quantity of chlorine added as potassium chloride, but all are lower than the theoretical quantity of chlorine, computed by considering the chlorine present in all the constituents of the mixture. Fertilizer chemists are aware that all the water-soluble potash put into a mixture cannot be recovered from the mixture by washing with water. Possibly chlorides are retained in the same way as potash. This seems to be the simplest explanation for the results that are less than the theoretical percentages present.

COLLABORATOR	MIXTURE NO. 1 pH = 6 THEORETICAL PERCENTAGE OF CHLORINE—2.55		MIXTURE NO. 2 pH = 4.8 THEORETICAL PERCENTAGE OF CHLORINE—3.55	
	Regular Method	With CaCO ₃	Regular Method	With CaCO ₃
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A. P. Kerr Baton Rouge, La.	2.34	2 32	3 22	3 27
W. F. Hand A. and M. College, Miss.	2 36	2 37	3 45	3 44
J. W. Kellogg Harrisburg, Pa.	2.17	2 22	3 08	3 07
H. P. Strack Nashville, Tenn.	2 18	2 21	3.18	3 21
R. Page Hudson Richmond, Va.	2.18	2 24	3 22	3 23
W. Catesby Jones Richmond, Va.	2.26	2 30	3 28	3 26
H. D. Reid Harvey, La.	2 32	2 38	3 45	3 44
L. V. Taylor Columbia, Mo.	2.35	2 37	3 30	3 34

RECOMMENDATIONS¹.

It is recommended—

(1) That the method given in this report for the direct titration of chlorine in mixed fertilizers be adopted as a provisional method (second reading).

(2) That the associate referee return to the study of methods for the determination of potash.

REPORT ON PLANTS.

By O. B. WINTER² (Agricultural Experiment Station, East Lansing, Mich.), *Referee*.

The work recommended to be done this year on plants was further study of methods for the following:

1. The preparation of plant materials for analysis.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12. 72.

² Presented by H. R. Kraybill.

2. The determination of total chlorine in plants.

3. The determination of some of the less common metals in plants.

No recommendation was made regarding the method for the determination of iron and aluminum in plants, and no cooperative work was called for during the year. The official method¹, however, is not satisfactory. Hence, considerable time was devoted by the referee to completing the work referred to by Patten and Winter² on a microchemical method by which iron and aluminum in plants could be precipitated as the phosphates, the iron and the aluminum separated, and each determined colorimetrically. The technic of the method was completed, and aluminum determinations were made on a number of plant materials. A paper describing the method will be submitted for publication later.

In the hands of three analysts in this laboratory the method gave results which fall within a range of approximately 5 per cent error. For this reason and also because it is suitable for the analysis of plant materials and there is a need for microchemical methods for the analysis of these materials, the referee suggests cooperative work on this method.

The results of the work on the preparation of plant material for analysis and the recommendations are given in the report of the associate referee.

Some collaborative work was done on the determination of total chlorine in plants, and some time was devoted to the wet digestion method. The results of this work and the recommendations are given in the report of the associate referee.

In the determination of the less common metals in plants, some collaborative work was done on manganese. The results of this work and the recommendations are given in the report of the associate referee.

RECOMMENDATIONS³.

It is recommended—

(1) That collaborative work be undertaken on a microchemical method for the determination of iron and of aluminum in plants.

(2) That consideration be given to the study of methods for the determination of carbohydrates and forms of nitrogen in plants.

(3) That the reports of the associate referees be accepted.

REPORT ON PREPARATION OF PLANT MATERIAL FOR ANALYSIS.

By H. B. KRAYBILL (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*.

During the past year studies were made of the suitability of the preservation in 80 per cent alcohol of fresh samples of plant material

¹ *Methods of Analysis*, A. O. A. C., 1925, 39.

² *This Journal*, 1928, 11: 207.

³ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 72.

for the determination of the more simple forms of nitrogen, such as ammonia and nitrate and amide nitrogen, and the determination of sugars. The work has not progressed sufficiently to justify recommendation with reference to adoption of methods.

It is recommended that study on the preparation of plant material for analysis be continued.

The Associate Referee on Less Common Metals in Plants gave an informal report in presenting his methods for manganese, copper, and zinc. No collaborative work had been done. The methods were adopted as tentative, and have been published¹. It was recommended that these methods be submitted to collaborators, and if found satisfactory that they be adopted as official methods.

REPORT ON TOTAL CHLORINE IN PLANTS.

By DORIS H. TILDEN (Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

It was recommended last year that collaborative work be undertaken on a method for the determination of chlorine in plant material, and that future work should ascertain whether organic chlorine can be determined by ashing. Owing to lack of time, only collaborative samples for the determination of chlorine in plant material were sent out.

This work was based on methods involving incineration, with sodium carbonate as a fixative for chlorine. The material used consisted of commercial sweetened pineapple juice that had been put up in glass bottles. The juice was filtered and sufficient formaldehyde was added to act as a preservative. It was then diluted so as to contain 10.5 per cent of total solids. One portion of this solution, to which a known quantity of chemically pure, recrystallized, dry sodium chloride was added, was designated as Sample I. Sample II was the diluted juice with no salt added. The methods sent to the collaborators are as follows:

PROPOSED METHOD FOR DETERMINING CHLORINE IN PLANT MATERIAL.

REAGENTS.

- (a) *Sodium carbonate solution*.—Dissolve 5 grams of sodium carbonate in water and make up to 100 cc.
- (b) *Silver nitrate solution*.—0.10 *N* or 0.05 *N*.
- (c) *Ammonium or potassium thiocyanate solution*.—0.1 *N* or 0.5 *N*.
- (d) *Ferric alum indicator*.

PREPARATION OF SAMPLE.

To the sample, or an aliquot of a sample solution containing not over 10 grams of organic matter and 100 mg. of chlorine, contained in a platinum dish, add 10 or 15 cc.

¹ *This Journal*, 1929, 12: 35.

of a 5 per cent sodium carbonate solution, evaporate to dryness, and char at a low temperature in a muffle. If necessary, add small quantities of water from time to time, evaporate, and heat further to get a white ash. (To avoid loss by decrepitation, it may be advisable to add a few cc. of alcohol to the dried residue and burn this off before reincinerating.) Dissolve the ash in dilute nitric acid and determine chlorine by the Gay-Lussac, gravimetric, or Volhard method.

PROCEDURE FOR GAY-LUSSAC METHOD.

Filter the nitric acid solution of the ash into a 750 cc. glass-stoppered Erlenmeyer flask, wash the filter thoroughly, and dilute to about 200 cc. with distilled water. Add from a buret an accurately standardized silver nitrate solution of a strength not over 0.1 *N*, and preferably about 0.05 *N*. (The silver nitrate solution may be added rapidly at first, but not in excess.) Stopper the flask, cover with a black cloth, and shake the solution violently after each addition until the precipitate becomes granular and leaves a clear supernatant liquid. When the precipitate becomes light, use great care to avoid passing the end point. Near the end of the titration hold the flask over a black glazed paper to aid in the detection of the end point. When the last two drops of the silver nitrate solution fail to produce a precipitate, take the previous reading as final and calculate the chlorine directly from the value of the standard solution.

NOTE.—This method requires much time toward the end of the determination owing to the necessity of allowing the silver chloride to settle completely after each addition of silver nitrate and also to the small quantity of precipitant that may be added each time.

PROCEDURE FOR GRAVIMETRIC METHOD.

To make a gravimetric determination, add about 1 cc. of silver nitrate in excess, shake, and filter after the solution has become clear. When a Gay-Lussac determination is not desired, proceed as for that method to the addition of dilute silver nitrate (not in excess of 0.1 *N* strength). Add the silver nitrate solution in *slight excess*, stopper the flask, cover it with a black cloth, and shake until the precipitate becomes granular and leaves an almost clear supernatant liquid. Allow to stand in the dark until clear, and then decant through a weighed Gooch crucible prepared with a thin pad of asbestos and previously dried at 140°–150°C. Wash by decantation several times, using in all 500 or 600 cc. of cold distilled water. Transfer the precipitate to the crucible with cold water, dry first at 100°C. and finally at 140°–150°C., weigh, and calculate the weight of silver chloride to chlorine. (The silver chloride becomes slightly discolored owing to exposure to light during the washing process, but a deep discoloration is due to the presence of occluded silver nitrate and indicates insufficient washing.)

PROCEDURE FOR VOLHARD METHOD.

Filter the ash solution into a 200 cc. glass-stoppered volumetric flask. Wash the filter, dilute, and add a measured excess of standard silver nitrate solution of 0.1 *N* strength or weaker. Stopper the flask and shake until the silver chloride becomes granular, make up to the mark with cold distilled water, mix, and allow to stand in the dark until the precipitate settles. Filter the entire solution through a dry filter paper, pipet 100 cc. of the filtrate into a clean flask, and titrate with standard 0.1 *N* thiocyanate solution and ferric alum indicator. Calculate the chlorine from the volume of the standard silver nitrate solution consumed.

The results received from collaborators are shown in Table 1.

TABLE 1.
Collaborative results on the determination of chlorine in two samples of pineapple juice.
 (Expressed as mg. per 100 cc.)

ANALYST	GAY-LUSSAC METHOD		GRAVIMETRIC METHOD		VOLHARD METHOD	
	Solution I*	Solution II	Solution I*	Solution II	Solution I*	Solution II
G. T. Daughters.....	. .	28.51	86.21	29.03	88.18	24.78
D. Dable.....	91.10	38.72	...	38.05	89.78	38.66
W. H. Kirby.....	90.60	33.0
H. R. Smith	88.5	31.2
E. L. Carmody	90.3; 89.9 90.1	31.3; 31.8 31.4; 31.4	89.7; 89.7 89.7; 89.0	30.6; 30.6 30.6; 30.6
D. H. Tilden (100 cc.)	87.49	26.27	90.69	27.31	85.38	33.52
(50 cc.)	92.30	33.84	96.72	33.34	92.62	
Variation	87.49 92.30	26.27 38.72	86.21 96.72	27.31 38.05	85.38—92.62	24.78—38.06
Average.....	90.29	31.83	90.65	31.70	89.32	31.50
Solution I minus Solution II.....	58.46	..	58.95	..	57.82
Theoretical	60.65	..	60.65	60.65

* Solution I is Solution II plus 60.65 mg. of chlorine per 100 cc.

DISCUSSION.

It is apparent from the figures given in the table that the variations arise from the sample itself, or the preparation of the ash, rather than from the methods of determination, for the general average of results indicates that the modified gravimetric, Gay-Lussac, and Volhard methods are about equally reliable and accurate. The Gay-Lussac method, although not practical from a general laboratory point of view, furnishes a means of checking, which is sometimes desirable. It will be noted that with the exception of one set of figures the results are lower on a 100 cc. than on a 50 cc. portion. Very possibly this is due to the larger amounts of solids (10.5 grams) for when 50 cc. was used the solids were about 5 grams. The results on the whole are disappointing, although those from a portion of the sample containing less than 8 grams (6-8) of organic solids hold considerable promise.

A sample solution of commercial, unsweetened pineapple juice, put up in tins, was prepared for collaborative work before the solutions finally sent out were prepared. In preliminary determinations difficulty was immediately encountered with the Gay-Lussac method. It was impossible to get a flocculent precipitate or any satisfactory settling out of silver chloride at any point in the determination. Sufficient silver nitrate was added to ensure an excess. The solution was boiled in an effort to coagulate the silver chloride and allowed to stand overnight, and an attempt was made to collect the precipitate on a Gooch crucible. This was entirely unsuccessful, however, for owing to its colloidal nature the silver chloride was not retained. After experimenting with sodium chloride solutions to which varying quantities of tin were added it was concluded that dissolved tin caused the trouble, and the solution was discarded. This occurrence, however, revived interest in wet ashing methods.

There are numerous references¹ to wet ashing methods for chlorine that have proved satisfactory under certain conditions. Most of the work, however, has been performed on physiological fluids and tissues, and plant tissues, where owing to the very small sample used little, if any, organic matter was present. In some cases the iodometric method was used², which, of course, is not suitable for the type of work under consideration.

Lawrence and Harris³, however, have worked on plant tissues with apparent success. Therefore, following their scheme, some experiments on the determination of chlorine by the dry ashing method were made on solutions prepared for collaborative work.

¹ Rehberg. *Biochem. J.*, 1926, 20: 483; Whitehorn. *J. Biol. Chem.*, 1920, 45: 449; Van Slyke. *J. Biol. Chem.*, 1923, 58: 523; Wilson and Ball. *J. Biol. Chem.*, 1928, 79: 221.

² Short and Gellis. *J. Biol. Chem.*, 1927, 73: 219.

³ *J. Am. Chem. Soc.*, 1924, 46: 1471.

Later, known solutions of recrystallized, chemically pure sodium chloride and pure cane sugar were used. On solutions of pure salt, without organic matter, very good results were obtained, but in its presence the results were unreliable. It is to be noted that Lawrence and Harris speak of removing *all* organic matter before proceeding with the back titration. This is easily done during the course of the short digestion period they recommend, where—as in plant tissues—there is very little organic matter in the form of carbohydrates present. Whitehorn, Van Slyke, and Rehberg also work with small quantities of sample, but they either have little organic matter present as carbohydrates, or they precipitate the protein substances with tungstic acid before digesting with nitric acid and excess of silver nitrate.

It was found that the sample solutions consisting of either a fruit juice or a known quantity of sodium chloride and sugar, with an excess of silver nitrate and nitric acid, cleared rapidly and gave an almost colorless solution and well-coagulated silver chloride. The nitric oxide fumes, however, caused so much trouble with the indicator (ferric alum) that an end point was not attainable. The rose color, indicative of the end of the reaction between the thiocyanate and silver nitrate, was fleeting and was only distinct with a large excess of thiocyanate. Even then it faded rapidly. Experiments were tried wherein the digested solution was reduced to a small volume—about 25–35 cc.—for the titration, but the result was the same unsatisfactory end point. Another aliquot was boiled with frequent additions of concentrated nitric acid and Perhydrol to aid in the digestion, but at the end of 2 hours enough organic matter remained to interfere materially. Diluting the digested solution to 200 cc. with distilled water and with dilute (50 per cent) boiled nitric acid gave no better results. A saturated solution of potassium permanganate was added to the cooled, slightly diluted, digested solution, just before the back titration was made, but with no success. A solution of hydrazine sulfate gave fairly satisfactory results. Diluting the solution to volume, after it had boiled long enough to coagulate the silver chloride, filtering off the precipitate, and titrating back the filtrate with potassium sulfocyanate gave a high back titration and consequently low results. An attempt to raise the boiling point of the digesting solution by adding potassium nitrate was a failure because when the solution became concentrated enough to aid in digesting the organic matter, salts crystallized out and charred.

From the meager results obtained, it appears that adding hydrazine sulfate to the sample solution—which has been boiled with concentrated nitric acid long enough to coagulate all the silver chloride and clarify the supernatant liquid—just before the back titration with potassium sulfocyanate is made and the indicator is added, gives the most promising results. Even at best, the ferric alum is acted upon in such a way that

an intense yellow color is immediately imparted to the solution and the end point is very delicate. There is, then, the necessity of removing all organic matter present if the wet ashing method for chlorine is to be used on plant materials usually met with in food analysis, or at least a procedure must be adopted to prevent the nitrogen peroxide fumes, even temporarily, from acting on the indicator.

SUMMARY.

1. Collaborative work was sent out for the determination of chlorine in plant material based on methods involving incineration with sodium carbonate as a fixative for chlorine.

2. Results obtained by collaborators indicate that the modified gravimetric, Gay-Lussac, and Volhard methods for the determination of chlorine are equally reliable and accurate.

3. Results show wide variations with the same sample solution, possibly due to the presence of too large an amount of organic solids.

4. Methods for the determination of chlorine in plant material involving wet ashing or the "open Carius method" have so far proved unsatisfactory owing to the difficulty in removing all the organic matter prior to making the back titration with potassium sulfocyanate.

RECOMMENDATIONS¹.

It is recommended—

(1) That further collaborative work be undertaken on methods for the determination of chlorine in plant material.

(2) That further work be done in investigating the possibilities of the "open Carius method" for the determination of chlorine in plant material.

(3) That future work include investigations to ascertain whether organic chlorine can be determined by ashing methods.

No report on dairy products was given by the general referee.

No report on butter was given by the associate referee.

REPORT ON CHEESE.

By E. O. HUEBNER (Dairy and Food Commission, Madison, Wis.),
Associate Referee.

No work was done this year owing to the pressure of other matters, but the associate referee wishes to call attention to two details of methods that are presented for action in the following recommendations:

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 72.

RECOMMENDATIONS¹.

It is recommended—

(1) That in the method for the quantitative determination of citric acid², line 19 should read as follows: "Hold at this temperature for 5 minutes and then add 25 cc. of the permanganate solution". The volume of permanganate solution, as stated in the method, is obviously insufficient.

(2) That in the description of the Schmidt-Bondzynski method for fat in cheese³, the sentence, "Add about 0.5 gram of sand to prevent bumping and boil gently for 5 minutes", be modified to read as follows: "Add about 0.5 gram of sand, previously digested with concentrated hydrochloric acid, and boil gently for 5 minutes". The suggestion does not modify the method in any way. It provides a means of avoiding difficulty with ferric chloride contamination of the fat. As most sands contain some iron, a provision should be made for its removal before the sand is used as directed in this method.

(3) That the recommendations for work adopted last year be repeated⁴.

No report on dried milk was given by the associate referee.

REPORT ON MALTED MILK.

By B. G. HARTMANN (Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

At the 1926 meeting of the association a preliminary report on the physiological identity of malted milk was presented⁵. The work was completed during this year, and two papers dealing with the chemical, physical and physiological identity and the microscopical identification of malted milk have been prepared for publication.

From the data presented in these papers it is concluded that the characteristics of malted milk are so well defined that no difficulty is experienced in differentiating this product from products of similar nature. The preparation of the papers prevented the associate referee from giving much time to the recommendation made last year relative to methods for determining the sugars contained in malted milk and its flavored products.

At the request of the referee, J. T. Keister of the Food, Drug and Insecticide Administration devoted some time to the determination of

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 77.

² *This Journal*, 1928, 11: 42.

³ *Methods of Analysis*, A. O. A. C., 1925, 279.

⁴ *This Journal*, 1928, 11: 76.

⁵ *Ibid.*, 1927, 10: 311

lactose in admixture with other sugars by the fermentation method. S. C. Rowe of the Food, Drug and Insecticide Administration also experimented with the fermentation method in connection with the determination of milk solids in milk bread. These analysts used the modified procedure proposed by Allen¹. Briefly, the procedure consists in fermenting the material under aeration and determining the residual lactose by copper reduction.

Keister and Rowe found that lactose in pure solution and in admixture with sucrose or pure dextrose (cerealose), when determined by the fermentation-copper reduction method, gives acceptable results. However, malt extract, commercial glucose, and commercial invert sugar leave a considerable residue after fermentation, which reduces copper. These latter observations are well established by Morris².

Apparently Allen's procedure is not suitable for the determination of lactose in malted milk or its flavored products. Keister conducted experiments for the purpose of determining whether the fermentable residue not lactose could be eliminated or reduced. Hydrolysis of the material with acid and diastase had no appreciable effect on the determination. A very decided reduction of the interfering residue was obtained, however, by fermenting with yeast that had been activated by incubating overnight at room temperature in a cerealose solution.

Owing to lack of time, the work could not be completed; however, judging from the results obtained it is believed that Allen's method, specifying activated yeast, shows promise of developing into a serviceable method for the determination of lactose in malted milk and its flavored products.

RECOMMENDATION³.

It is recommended that the study of methods for the determination of lactose in malted milk be continued.

No report on ice cream was given by the associate referee.

No report on milk proteins was given by the associate referee.

No report on qualitative tests was given by the associate referee.

¹ *Proc. World's Dairy Congress*, 1923, 2: 1316.

² *J. Fed. Inst. Brew.*, 1898, 4: 162.

³ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 75.

REPORT ON FATS AND OILS.

By G. S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

During the past year, in accordance with the recommendations, collaborative study was continued on the lead-salt-ether method for the determination of saturated and unsaturated acids. Also a study was made of the "cold test" method as applied to commercial salad oils.

Since the lead-salt-ether procedure has been described in detail and published¹, it is not necessary to repeat it. The cold test has also been published².

The results of the determination of saturated and unsaturated acids by the lead-salt-ether method are given in Table 1. Sample No. 1 was a refined wintered cottonseed oil; No. 2 was a refined corn oil; and No. 3 was a crude peanut oil.

The results obtained this year and last year with the lead-salt-ether method confirm previous observations, namely, that extensive experience is required on the part of the analyst before the difficult technic can be mastered. In view of this fact, the results with some obvious exceptions show fair agreement. After a careful examination of the results reported for the past two years, it is concluded that little or nothing is to be gained by continuing a collaborative study on this procedure for another year, because sufficient work has been done to demonstrate that when the directions are actually followed in every detail as given satisfactory results are obtained.

It is suggested that this method be substituted for the old tentative method, which is entitled "Liquid and Solid Fatty Acids"³, because the latter is not only incomplete in the description of the lead-salt-ether procedure, but it lacks many important details of the proper technic. It is recommended that the method be made official.

It should be noted that McKinney and Gertler used the Hanus method, while the other collaborators used the Wijs method for the determination of the iodine numbers given in Table 1.

The results obtained with the cold test are given in Table 2.

In view of the satisfactory results obtained with the cold test, it is recommended that the method be not studied further, and that it be made official.

It is recommended that next year a study be made of the combined procedure for the determination of the Reichert-Meissl and Polenske values, which is now quite commonly used in place of the separate procedures described in *Methods of Analysis*.

¹ *This Journal*, 1928, 11: 301; 1929, 12: 44

² *Ibid.*, 1929, 12: 46

³ *Methods of Analysis*, A. O. A. C., 1925, 292

TABLE
Collaborative results with

ANALYST	SAMPLE 1					
	SATURATED ACIDS AS DETERMINED	IODINE NUMBER	SATURATED ACIDS CORRECTED	UNSATURATED ACIDS AS DETERMINED	UNSATURATED ACIDS CORRECTED	IODINE NUMBER UNSATURATED ACIDS
P. A. Sigler	<i>per cent</i> 21.66	4.7	<i>per cent</i> 20.91	<i>per cent</i> 72.96	<i>per cent</i> 73.71	134.0
	22.08	4.4	21.34			130.5
A. Edeler	26.18	26.9	21.4	68.92	73.7	147.2
	25.92	26.8	21.2	69.54	74.3	147.0
R. S. McKinney	21.62	4.4	20.93	73.38	74.07	140.8
	22.13	7.3	20.98	73.37	74.52	139.9
S. I. Gertler	20.07	5.2	19.25	73.55	74.37	132.3
	20.18	5.3	19.33	73.64	74.49	132.5
W. D. Richardson Analyst No. 1	23.31	24.9	19.46	68.11	71.96	150.6
	23.06	20.1	19.97	68.73	71.82	149.7
Analyst No. 2	23.46	9.8	21.93	69.88	71.41	149.5
	24.58	15.3	22.06	67.92	70.44	149.2
M. L. Sheely	22.01	17.2	19.32	71.36	74.05	140.5
	20.28	6.6	19.26	72.92	73.94	131.2
F. Fenger Analyst No. 1	21.90	6.8	20.91	73.00	73.99	150.1
	21.00	7.1	20.10	73.50	74.48	149.5

TABLE 2.

Cold test.

ANALYST	SAMPLE	APPEARANCE
F. Fenger	1	Clear and limpid
	2	Clear and limpid
	3	Solidified
W. D. Richardson	1	Clear and limpid
	2	Clear and limpid
M. L. Sheely	1	Partially solidified
	2	Clear and limpid
	3	Solidified
R. S. McKinney	1	Clear and limpid
	2	Clear and limpid
	3	Solidified
A. Edeler	2	Solidified
	3	Solidified

1.

the lead-salt-ether method.

SAMPLE 2						SAMPLE 3					
SATURATED ACIDS AS DETERMINED	IODINE NUMBER	SATURATED ACIDS CORRECTED	UNSATURATED ACIDS AS DETERMINED	UNSATURATED ACIDS CORRECTED	IODINE NUMBER UNSATURATED ACIDS	SATURATED ACIDS AS DETERMINED	IODINE NUMBER	SATURATED ACIDS CORRECTED	UNSATURATED ACIDS AS DETERMINED	UNSATURATED ACIDS CORRECTED	IODINE NUMBER UNSATURATED ACIDS
<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
9.72	13.7	8.72	85.13	86.13	132.2	20.03	5.8	18.93	73.58	74.68	105.0
9.08	11.5	8.27	85.91	86.72	127.7	19.60	4.3	18.71	74.15	75.04	99.4
9.23	23.4	7.8	85.73	87.2	149.2	23.59	16.7	20.2	70.12	73.5	116.6
8.20	17.7	7.2	86.87	87.9	144.9	22.2	11.7	19.9	71.91	74.2	115.3
8.59	7.6	8.11	86.46	86.94	136.9	20.02	6.4	18.88	73.26	74.70	111.5
8.75	7.8	8.29	86.44	86.90	147.1	19.94	5.9	18.92	73.82	74.85	115.1
8.45	8.4	7.89	86.04	86.64	135.9	20.41	6.2	19.21	72.07	73.27	112.5
9.59	10.2	8.87	85.29	86.01	136.7	20.69	6.3	19.44	72.41	73.66	110.9
9.76	24.6	8.14	81.88	83.50	148.4						
9.51	23.0	8.04	81.73	83.20	148.8						
9.59	14.8	8.64	82.11	83.06	149.2						
10.45	13.9	9.48	82.06	83.03	149.2						
7.58	11.4	6.96	86.71	87.33	139.2	22.17	22.0	17.43	70.26	75.00	103.0
8.52	17.7	7.44	85.13	86.21	139.0	19.90	11.4	17.76	73.07	75.21	106.1
7.95	10.5	7.39	85.50	86.10	149.7	19.50	6.34	18.47	73.67	74.68	117.6
9.07	14.95	8.17	84.90	85.80	150.0	20.25	7.52	18.94	72.70	74.01	116.5

RECOMMENDATIONS¹.

It is recommended—

- (1) That the cold test be made official.
- (2) That the lead-salt-ether method be made official.
- (3) That a study be made of the combined procedure for the determination of the Reichert-Meissl and Polenske values.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 79.

REPORT ON BAKING POWDERS AND BAKING CHEMICALS.

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.),
Referee.

In 1928 the work on baking powder was confined to the direct determination of aluminum in a combination baking powder.

The referee first studied a volumetric method proposed by A. Wöhlk¹. The method depends on the formation of the complex salt potassium-aluminum-oxalate, which is neutral in solution. Aluminum hydroxide is dissolved in standardized oxalic acid, and the excess of oxalic acid is titrated with sodium hydroxide or borax. The results obtained by the referee were unsatisfactory.

Next, the referee studied a method proposed by Hess and Campbell². With some slight modifications this method was found to be quite satisfactory, and it was submitted to collaborative study. It is as follows:

Method I.

ALUMINUM.

REAGENTS.

(a) *Ammonium bisulfite solution.*—Pass sulfur dioxide into a cool, dilute solution of ammonia (1 + 1) until the color of the solution becomes distinctly yellow.

(b) *Phenylhydrazine bisulfite solution.*—To a few cubic centimeters of phenylhydrazine add gradually a saturated solution of sulfur dioxide until the precipitate of phenylhydrazine sulfite, which at first separates out in crystals, is almost redissolved. If the precipitate is completely dissolved, add a drop or two of phenylhydrazine until a slight precipitate of phenylhydrazine sulfite is obtained. Filter the solution before using. (From 5 to 10 cc. of this solution to 100 cc. of water is sufficient strength for washing the alumina precipitate. This concentrated solution of phenylhydrazine bisulfite, if well stoppered, will keep indefinitely.)

Dilute hydrochloric acid.—Add 10 volumes of water to 4 of strong hydrochloric acid.

DETERMINATION.

Ignite 3 grams of baking powder at a temperature not exceeding 550°C. As soon as the carbon has burned off, take up the residue in hydrochloric acid (4 + 10) and boil gently to assist solution. Filter into a 300 cc. volumetric flask and wash with hot water. Ignite the insoluble residue and filter paper in a platinum crucible and then fuse the residue with about 2 grams of sodium carbonate. Dissolve the fused mass in water and hydrochloric acid and transfer to the volumetric flask containing the original filtrate. Cool, and make up to volume.

Transfer 100 cc. aliquots to 400 cc. beakers. Heat nearly to boiling, add dilute ammonium hydroxide until a slight permanent precipitate forms, then just redissolve this precipitate with a drop or two of dilute hydrochloric acid. Add, drop by drop with constant stirring, 10 or 12 drops of a saturated solution of ammonium bisulfite. Then add to the hot solution sufficient phenylhydrazine to precipitate the alumina completely (1 or 2 cc. is generally enough; an excess colors the solution yellow). If a permanent precipitate does not form at this point, add dilute ammonia carefully, drop by drop, just to a permanent precipitate, and then complete the precipitation

¹ *Dansk Tidss. Farm.*, 1927, 1: 525; *Brit. Chem. Abstracts*—A, Jan., 1928

² *J. Am. Chem. Soc.*, 1899, 21: 776

by adding a few more drops of the phenylhydrazine. Let stand a few minutes for the precipitate to settle and filter while still warm. Wash the precipitate with warm water containing the phenylhydrazine bisulfite until the washings give no test for iron when yellow ammonium sulfide is added.

Place the filter paper containing the precipitate in a weighed platinum crucible. Dry, char, and ignite at a low temperature. After the filter paper has completely burned, continue the ignition at a bright red heat to constant weight. Weigh quickly with the cover on the crucible, as the precipitate is very hygroscopic. (A second weighing is always necessary.) The precipitate consists of aluminum oxide and aluminum phosphate.

Fuse the ignited precipitate with about 2 grams of sodium carbonate and dissolve the fusion in dilute nitric acid (1 + 9). Transfer to a 250 cc. beaker, and boil to insure that all the phosphoric acid is in the ortho state. Cool. Transfer to a 200 cc. flask, make up to volume, and use 50 cc. aliquots to determine the phosphorus pentoxide. Multiply the weight of phosphorus pentoxide obtained by 4 and subtract the product from the weight of combined precipitates obtained above. The difference is the weight of aluminum oxide in 1 gram of baking powder.

Weight of $\text{Al}_2\text{O}_3 \times 100 =$ percentage of Al_2O_3 .

Percentage of $\text{Al}_2\text{O}_3 \times 4.749 =$ percentage of $\text{Na}_2\text{Al}_2(\text{SO}_4)_4$.

If the baking powder contains a significant quantity of silica, remove it by evaporating the hydrochloric acid solution of the powder to dryness and dehydrating at 105°C . for 2 hours. Add to the dry mass 10 cc. of hydrochloric acid and 100 cc. of water, boil, filter off the silica, and proceed as in the method described above.

Method II.

An approximation of the quantity of S. A. S. present may be obtained by determining the sulfates on an aliquot of the hydrochloric acid solution of the baking powder. Determine the sulfates by the method given in *Methods of Analysis*, A. O. A. C., 1925, p. 45, par. 17. $\text{BaSO}_4 \times \text{the factor } 0.5185 = \text{Na}_2\text{Al}_2(\text{SO}_4)_4$. The sulfate results will usually be high owing to the presence in the baking powder of sulfates other than sodium aluminum sulfate.

From samples of baking powder sent to collaborators the following results were obtained by the methods submitted:

	Al_2O_3	METHOD I		METHOD II
	per cent	$\text{Na}_2\text{Al}_2(\text{SO}_4)_4$ from Al_2O_3		$\text{Na}_2\text{Al}_2(\text{SO}_4)_4$ from BaSO_4
		per cent		per cent
C. B. Morison, American Institute of Baking, Chicago, Ill.	4.33 (av.)	20.56		
R. Kreyling, Provident Chemical Works, St. Louis, Mo.	3.90 3.82	18.52 18 14	} 18 33	19 20 19 20
— Wright, Royal Baking Powder Co., Brooklyn, N. Y.	4.23 4.18 4.16	20.08 19.85 19.76		19 90
E. W. Thornton, Davis Baking Powder Co., Hoboken, N. J.	4.77	22.65		
Milton H. Kemp, Calumet Baking Powder Co., Chicago, Ill.	4.24 4 29	20 14 20 37	} 20 26	20 55 20 42
Percy O'Meara, Department of Ag- riculture, Lansing, Mich.	4.63 4.68 4.57	21 95 22 20 21 70		21 95
L. H. Bailey	4 06 4 11	19 28 19 52	} 19 40	19 33 19 50

From the results obtained it is recommended¹ that the method of precipitating the alumina by phenylhydrazine be adopted as a tentative method and that further collaborative work be done on it.

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4. KOLTHOFF AND SANDELL. Rapid Method for the Separation of Aluminum and Beryllium. *J. Am. Chem. Soc.*, 1928, 50: 1900.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 79.

CONTRIBUTED PAPERS.

JENA GLASS FILTERING CRUCIBLES¹.

By R. H. SIMON (Agricultural Experiment Station, Wooster, Ohio).

The development of the Jena glass filter plate described by Huttig² has made possible a filtering unit which should be included in the usual laboratory filtering apparatus. This plate of sintered glass, which is a rigid part of the crucible or tube, filters equally well with vacuum or pressure. A plate of suitable porosity makes possible the rapid filtration of fine and troublesome precipitates. The purity of the precipitate may be determined from its general appearance on the plate during the washing process following its filtration. For precipitates that are dried to constant weight the determination is finished quickly since the glass crucible may be wiped dry and very little moisture remains in the porous glass plate. Should reprecipitation be necessary, the precipitate is easily dissolved and washed into the original beaker, or if the determination is finished volumetrically, the crucible and contents may be placed into the measured standard solution. The end point of the back titration is not obscured by the crucible or filtering medium. The filter is a rapid, efficient, and clean piece of apparatus.

Adaptation to Common Filtering Apparatus.—These crucibles do not require special equipment since a rubber filter holder supports the crucible in a funnel, thus making a glass to glass connection that insures complete recovery of a dissolved precipitate when a second precipitation is necessary. A crucible may be removed easily and wiped dry for such precipitates as may be determined by difference in weight, such as potassium. A crucible of 15 cc. capacity, with a plate porosity of less than 7 ($4-5\ \mu$) is suitable for the filtration of phosphorus and it is light enough to be used for the small quantities of potassium that usually occurs with phosphorus, as in plant materials. The crucible thus supported in a funnel is easily adapted to any suction flask or bell jar for vacuum filtering.

PHOSPHORUS DETERMINATIONS.

Phosphorus precipitated according to the method of Ames and Boltz³ filters rapidly through the plate of $4-5\ \mu$ porosity with very little suction. If the quantity of phosphorus is small, a second precipitation, in which 2 cc. of ammonium molybdate is used, may follow. After the precipitate is washed, the greater part (bulk) is transferred from the crucible to the original precipitating vessel with a stream of water. The

¹ Contribution from the Ohio Agricultural Experiment Station, Wooster, Ohio.

² *Z. angew. Chem.*, 1924, 37: 48; and Nette, *Z. anal. Chem.*, 1925, 65: 385.

³ *Ohio Agr. Expt. Sta. Bull.* 285 (1915), p. 206.

crucible is then placed in this vessel with the measured excess of standard hydroxide, and the completeness of the solution is easily observed. The end point of the titration is sharp because there is no filtering medium to interfere.

When 10 cc. of a solution of ammonium phosphate for each determination was used, the mean of a set of 12 determinations was 43.66 ± 0.149 mg. of phosphorus. A second set of 12 determinations from the same solution gave a mean of 43.62 ± 0.154 mg. of phosphorus. By comparing the means of each set it is possible to duplicate the determinations with accuracy. Should the highest determination of each set be eliminated, the mean of each set becomes 43.57 mg., with a probable error on a single determination of ± 0.149 mg. and ± 0.154 mg., respectively. The accuracy of the glass crucibles is apparent when these results are compared with those obtained with Shimer¹ filter tubes of micro capacity. A set of 10 determinations made with Shimer filters from the same solution of ammonium phosphate gave a mean of 43.56 ± 0.194 mg. of phosphorus. The glass crucibles are always ready for use, while the Shimer filter requires a filter pad and complete assembly for each determination. This evidence of accuracy and saving of time makes a favorable comparison with apparatus that has been accepted as standard equipment. Adaptation to a common filtering apparatus, as well as for the determination of other elements have given these filters a definite place in laboratory equipment.

POTASSIUM DETERMINATIONS.

The time for the tedious process of determining potassium can be shortened by the use of the Jena glass crucibles because the salt may be dried immediately in the crucible, and a direct weight by difference finishes the determination. The position of the plate above the bottom of the crucible insures a quick escape of the last traces of alcohol and avoids the possibility of reducing the precipitate. When a filter pad of asbestos is used in a Gooch crucible the alcohol must escape at low temperatures, and likewise more time is required for its escape from the filter tube described by Schollenberger². When this tube is used traces of alcohol may be carried into the weighed dish when the salt is dissolved and reduction will occur when the solution is evaporated. If the determination is finished directly by difference in weight when a Gooch is used, the reduction of the salt is avoided, but there is always a chance of a loss of the asbestos filtering medium. By using a glass crucible that is constant in weight, the determination is finished with certainty. For small weights of the salt a double weighing is necessary, but for larger quantities the weight of the crucible may be considered as constant.

¹ *J. Am. Chem. Soc.*, 1905, 27: 287.

² *J. Ind. Eng. Chem.*, 1912, 4: 436.

Twenty determinations were made from a solution of potassium sulfate. The mean was 76.8 ± 0.61 mg. of potassium chloroplatinate. For a more dilute solution the mean was 27.4 ± 0.22 mg. of potassium chloroplatinate. When the probable error of 0.61 mg. and 0.22 mg. of potassium chloroplatinate is reduced to potassium it becomes 0.098 and 0.035 mg., which is a lower error than that obtained from tubes or Gooch crucibles with asbestos as the filtering medium. Besides, the large filtering area of the plate insures the continued use of the crucible for a long period.

Very little trouble was encountered with these crucibles when used for phosphorus determinations, as the hydroxide dissolves all the precipitate. In potassium work, however, the accumulation of residue insoluble in hot water decreases the porosity. Hot water with vacuum will dissolve this residue from the plate easily when the crucible is inverted in the filter holder. For more thorough treatment the crucibles are boiled in aqua regia and then washed by hot water with vacuum. With careful use no loss by breakage should occur.

CONCLUSION.

Since their introduction glass crucibles of porosity less than 7 ($4-5 \mu$) have been used successfully by the writer in determinations of phosphorus and potassium. Larger porosity has been used for potassium. The fixed plate in a glass crucible is an ideal unit for volumetric determinations. Much time is saved by its use in the determination of potassium. Crucibles may be cleaned and easily restored for use. If reasonable care is taken these filtering units are most economical.

THE RELATION OF RESEARCH TO CONTROL¹.

By C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.).

Legal enactments for controlling the purity of foods, drugs, and other commodities are as old as the written records of mankind. It is impossible to determine the date of the first law to curb adulteration, which as a social offense goes back to the time when the needs of the human race impelled individuals to draw nearer for self-protection and then, with the tribal division of occupations, to entrust to their neighbors some portion of the work which they formerly performed for themselves. The addition of foreign seeds to the grain which was to be used for food and the mingling of wood ashes with the meal which was to be baked on the hearth-stone were perhaps the first crude offenses of this character,—

¹ Presented October 20, 1926, before the joint meeting of the Association of Official Agricultural Chemists, the Association of Dairy, Food and Drug Officials of the United States, and the Association of Feed Control Officials of the United States in celebration of the 20th anniversary of the passage of the Federal Food and Drugs Act.

ignorantly done no doubt at first, but afterwards, when barter was introduced, intentionally performed and then, with the increasing complexity of civilization, more and more craftily concealed. Whatever may have been the course of its prehistoric development, we find in the first written records of legislation specific enactments against the practice of adulteration.

We may be very certain also that in this prehistoric period man was exercising his perceptive faculties in every possible way to avoid being deceived. The appearance, odor, and taste of the suspected article were compared with those of the genuine, the tests employed being all of the so-called organoleptic character. Then came observations upon the behavior of substances when burned, winnowed, or thrown into water, and finally upon the changes that took place when products were treated with other substances,—the knowledge acquired in this way constituting the first beginnings of chemical analysis. There were thus developed in this long prehistoric period, many thousands of years in duration, the first rudimentary relations of research to control. Methods were very crude and they remained so until after the invention by primitive man of his first tools of research, which were simple devices for the measurement of length, volume, and weight. These inventions, which in their final perfected form were of the greatest service in raising mankind from barbarism to civilization, were mechanical outgrowths of the natural uses that were made by early man of his hands and feet. A measuring stick, divided into foot lengths, was easier to apply than the heel to toe method of estimating distance, which might be modified to suit the desire of the user. An empty gourd, as a measure of volume, was more convenient to use than a curved-up hand, besides having the additional advantage of not being susceptible to modification, and two such gourds attached by thongs to a stick suspended in the middle was a more impersonal and truthful method of determining weight than the hefting of two objects in the opposite palms. Man's need of liberating himself from the deceptions and limitations of subjective methods gave rise to the measuring rod, the measuring vessel, and the balance.

The sharpening and stimulation of man's faculties in his effort to avoid deception have played a great part in his intellectual advancement, and to this extent civilization is indebted to the adulterator and counterfeiter. Many examples of this could be given, but I shall limit myself to a few illustrations which are of historic interest in showing the relationship of research to control.

The first illustration relates to the discovery of the principle of specific gravity which is used so extensively by control chemists at the present day as a means of detecting adulteration. The discovery was made by the Greek mathematician Archimedes in the third century B. C. and was the direct result of the attempt of a metal worker to adulterate gold.

King Hiero of Syracuse gave this metal worker a weighed amount of pure gold with the commission to have it made into a crown. When the work was finished, the workman gave the crown to the king, who found it to weigh exactly the same as the original amount of fine gold. The king suspected, however, that a portion of the gold might have been abstracted and replaced by a baser metal and accordingly submitted the matter to Archimedes for a decision. The latter puzzled over the problem until one day, as he was stepping into a bath tub which had been filled to the brim, he observed the overflow of water displaced by his body. The solution of the problem immediately flashed through his mind for he perceived that the excess of volume occasioned by the introduction of a lighter metal could be determined by putting the crown and an equal weight of fine gold successively into a vessel filled with water and measuring the amounts of liquid displaced. Archimedes was so overjoyed at this happy thought that, forgetting his clothes, he rushed home exclaiming "Heureka, Heureka, I have found it". He immediately put his idea to a test and found the crown, as the king had suspected, to be adulterated. The discovery of the principle of specific gravity was thus the direct result of a regulatory mandate. The joy which Archimedes felt in making his discovery was not, however, the fact that he had detected a criminal but because he realized the importance of what he had found as a contribution to pure science.

It is a jump of 1900 years from Archimedes, the first to employ the exact methods of scientific research for detecting adulterations, to Robert Boyle, who has been called the father of modern chemistry. Boyle greatly extended the application of the hydrostatic principle, discovered by Archimedes, to determining the purity of various substances used in medicine. The results of his work were published in 1690 in a book called "*Medicina Hydrostatica*" or "*Hydrostatics Applied to the Materia Medica*", which has been called the first treatise ever written with the object of giving exact scientific information upon methods for detecting adulteration. His publication was of immense value in its description of processes that could be used for pure research although it was written with the primary purpose, announced upon the title page, of "shewing how, by the Weight that divers Bodies, used in Physick, have in Water, one may discover Whether they be Genuine or Adulterate". We have thus in the case of Boyle another illustration of the important mutual relationship of research and control.

A Dutch contemporary of Boyle, named Leeuwenhoek, was at this time developing another very important tool of scientific research which was destined to serve in later years as one of the most important weapons in the warfare against food and drug adulteration. This tool of research was the microscope, and the discoveries which Leeuwenhoek made with this instrument upon the minute cellular structure of plant and animal

tissues were of the greatest importance for they revealed the possibilities of investigation in this field. Leeuwenhoek, however, was too interested in research to make use of the microscope as a means of detecting adulteration. In fact, this application of the microscope was largely overlooked during the next two centuries until an English physician, Dr. Arthur Hassall, in 1850, began to make a scientific study of the cellular structure of the coffee bean. Hassall then compared this structure with that of chicory, corn, beans, and other substances that were used for adulterating coffee and noted that each one of these substitutes had a different microscopic appearance. This observation was of the greatest value, for chemists had been searching in vain for an accurate method for determining the purity of ground coffee. Hassall's publication attracted immediate attention, and he was commissioned by the "Lancet", an English medical journal, to extend his investigations to cocoa, pepper, and many other articles of food. Hassall's work as chairman of the Lancet Analytical Sanitary Commission led to such startling disclosures that in 1860 the English Adulteration of Food and Drink Act was passed, the first law to deal generally with the adulteration of food and the parent law of all similar legislation in other countries.

Hassall's early work was research of the highest order, and had he been interested only in the purely scientific phases of his subject the passage of the Adulteration of Food and Drink Act would have been much longer postponed. But as a physician he was greatly concerned with the health and welfare of the public and foregoing the attractions of pure research devoted himself unsparingly to the initiation of a great reform. He had the rare double qualifications of the scientific investigator and the courageous regulatory official, a combination for which the world had waited many centuries. His life work is an outstanding illustration of the importance of research to regulatory control.

The work of Dr. Hassall in England was contemporaneous with some early investigations of a somewhat similar character that were conducted by a physician in the United States. I refer to the researches upon adulteration conducted by Dr. Lewis C. Beck of Rutgers College, who published a book in 1846, entitled "Adulterations of Various Substances Used in Medicine and the Arts". This book dealt not only with the adulteration of medicines but with that of foods and described the methods for detecting the presence of impurities in such products. Previous writers had dealt with the adulteration of foods and drugs in the United States, but Dr. Beck was the first to apply the exact methods of chemical research to regulatory problems. There were in existence in Dr. Beck's time numerous state inspection laws for protecting the public against the adulteration of potash, flour, beer, wine, drugs, and other commodities. Many of these inspection laws dated back to Colonial time, but as a means of protecting the public against fraud and deception

they were almost valueless because no scientific research had been conducted upon exact methods for detecting and controlling adulteration. In 1832 the Committee on Trade and Manufactures of the New York Legislature commissioned Dr. Beck to investigate the processes of manufacturing potash and to make analyses of samples of potash purchased on the market. The report submitted by Dr. Beck upon this subject is the earliest illustration of the application of research to control work in the United States. In the words of Dr. L. F. Kebler, who has made a study of Dr. Beck's life and work, the report "is deserving of study by anyone engaged in regulatory work. The investigation covered patents, manufacturing operations, injury to trade caused by adulterated products, analytical data, a standard for pure potash, and necessary legislation".

The Federal Government did not begin to be interested in human foods until 1848, when the 30th Congress appropriated one thousand dollars "for defraying the expenses of chemical analyses of vegetable substances produced and used for the food of man and animals in the United States, to be expended under the direction of the Commissioner of Patents". The Commissioner of Patents selected as the chemist to conduct this investigation, Dr. Beck, whose "Report on the Breadstuffs of the United States" covers 29 pages of the Patent Office Report upon Agriculture for the year 1848 and 34 pages of the similar report for 1849. After describing his methods for analyzing flours and for detecting the presence of adulterants in this report, Dr. Beck makes some very important observations upon the best means of controlling fraud and of improving the character of American breadstuffs. The report is a splendid piece of practical scientific work which must always be referred to by American chemists. Dr. Beck's pioneer investigations paved the way for future research upon food and drug control in the United States.

There is not time to sketch in detail the development of regulatory chemical work in the different States of the Union. The States anticipated the Federal Government in this as in many other lines of law enforcement. It seems to us at the present day as rather irrational that agricultural commodity regulation should have taken up fertilizers first, then cattle feeds and finally human foods, yet such was the sequence which was followed in nearly all the States. It will also be recalled that the methods of our Association of Official Agricultural Chemists were devoted exclusively to fertilizer analysis during the first few years and that methods of analyzing human foods were not taken up until much later. Maryland seems to have been the first State that passed a fertilizer control law and the execution of this law devolved upon the State agricultural chemist, an office that Maryland seems also to have had the honor of first creating, in 1848. Dr. James Higgins, the first appointee, also conducted a large amount of agricultural chemical investi-

gation during his tenure of this office, the double functions of research and control apparently causing him no serious inconvenience. Other States followed Maryland's example and the office of State agricultural chemist continued down to the time of the establishment of the State colleges and experiment stations, when it was gradually abolished.

The agricultural experiment stations have exerted a very wide influence in controlling the purity of fertilizers, feeding stuffs, insecticides, and human foods in the different States, their work in this respect filling a large part in the history of agricultural commodity regulation in America. Yet the primary function of these stations, as indicated by their title, was experiment and not control. In the early days the chemists of the experiment stations were the ones best qualified to make analyses of the commodities consumed, or produced, by agriculture and having in many cases been the ones to initiate State regulatory laws, the work of chemical control fell naturally at first to them. The fees for such analyses also constituted a considerable source of income to the experiment stations which in this early period, when financial aid was especially needed, were glad to obtain funds for the support of research, although it was soon recognized that the growing demands of regulatory work seriously interfered with agricultural investigations.

The relations of research to the control of foods, drugs, feeding-stuffs, insecticides, etc., by the Federal Government have been of a somewhat different character than those observed in the individual States. The same general outgrowth of regulatory work from pre-existing research took place, the same mutual benefits and interferences of these two lines of activity occurred, and the same ultimate tendency towards segregation has developed, these various stages of evolution being, however, slower but much more extensive in degree.

Agricultural chemical research under the auspices of the Federal Government had its first inception in the passage by Congress in January 1830 of a resolution for the Secretary of the Treasury to conduct an inquiry upon the cultivation of sugar cane and upon the fabrication and refinement of sugar. In August, 1832, Dr. Benjamin Silliman of Yale University was engaged to conduct this investigation and his report of 122 pages, prepared with the assistance of several collaborators, was printed in May, 1833. This research, it will be noted, was not conducted by a regularly paid Government official, but by an outside scientist, and this was the method employed in all agricultural chemical investigations initiated by the Government until the establishment of the present Department of Agriculture in 1862. In 1842 the general direction of such investigations began to be supervised by the Commissioner of Patents, whose annual reports upon agriculture during the next twenty years contain many contributions by specially engaged chemists upon agricultural chemical subjects. Many of these papers, such as the one

already mentioned by Dr. Beck upon the breadstuffs of the United States, had an important bearing upon future regulatory operations by State and Federal authorities. In addition to the paper by Dr. Beck upon cereal products, the reports by William Webb of Delaware in 1842 and by John Beal of Indiana in 1843 upon maize sugar, by Justus Liebig of Germany in 1845 upon artificial manures, by Nicholas Longworth of Cincinnati in 1847 upon native wines, by C. L. Fleischmann in 1848 upon cane sugar, by J. H. Salisbury of New York in 1849 upon the chemical composition of Indian corn, and in 1850 upon the chemical composition of American apples, by J. P. Norton of Yale College in 1851 upon mineral manures, by J. C. Booth of Philadelphia in 1852 upon soil analysis, by C. T. Jackson of Boston in 1855 upon cottonseed products and in 1857 upon sorghum, and by T. G. Clemson of South Carolina in 1859 upon fertilizers, may be mentioned as a few examples of the contributions by eminent chemists that were published in the annual reports of the Commissioner of Patents. These reports are all outstanding illustrations of valuable chemical research, and their pages contain frequent references to the importance of a regulatory supervision of the products which are employed or produced by agriculture.

In 1862, when the Department of Agriculture was established, Dr. C. M. Wetherill was chosen to direct the Government's agricultural chemical operations. But the opportunities for chemical research during the first ten years of the Department's history were very few and far between. The chemist of the Department had a poorly equipped laboratory with little or no assistance, and his time for the most part was frittered away in examining numerous miscellaneous substances such as minerals and ores which the curious-minded farmers of the country picked up on their land and sent in to the Department for analysis. A Government chemist was looked upon at that time, as he is regarded frequently even now, as an agent for rendering gratuitous chemical services to whomsoever might ask. He was exploited by selfish industries, who wished to get something for nothing, and we accordingly find Dr. Antisell, the chemist of the Department from 1866 to 1870, complaining that he experienced great difficulty in trying "to avoid subserving private interest while seeking public good". Yet with all these difficulties the early chemists of the Department made a splendid preliminary chemical survey of the agricultural resources of the United States with regard to soils, fertilizers, cereals, root crops, tanning materials, sugar producing plants, fruits, and various human and animal foods. Such materials as disinfectants, pharmaceuticals, mineral waters, and food poisons were also investigated and one notes a frequently expressed desire on the part of the Department chemist to protect the farmer from fraud, but without the necessary Federal legislation there was not much that he could accomplish. The most that Dr. Antisell could advise the farmers 'to

do, if they wished to avoid deception in the purchase of fertilizers, was to calculate long in advance the total needs of the year and then to buy all their requirements at once of the same lot in the early spring before the output of the factories began to depreciate in quality. "This is the only way", wrote Dr. Antisell, "by which the agricultural community can protect itself against the impositions of trade".

In 1883 Dr. Harvey W. Wiley was appointed Chief Chemist of the Department and it is from the work which was developed under his direction that we can draw our best illustrations of the importance of research to chemical control. The chemical research work of the Department was directed by Dr. Wiley along three major lines of activity: first, researches upon the sugar cane and sugar beet; second, improvements upon methods of agricultural analyses; and third, investigations upon human foods. Each one of these subjects, if properly treated, would occupy the entire session of our program. The fundamental researches upon the sugar cane and sugar beet, conducted by Dr. Wiley and under his direction by such chemists as Spencer, Crampton, Edson, Maxwell, and Bryan, are published in some thirty bulletins of the Bureau of Chemistry. This work had an important bearing upon certain future regulatory developments, more especially as regards the setting of standards for sugar, sirups, and molasses, but it is of too special a character for discussion at this time.

The researches conducted by Dr. Wiley and his associates upon the improvement of chemical methods for the analysis of fertilizers, insecticides, feeding stuffs, foods, and drugs have been of the utmost importance, for upon these early investigations accurate methods were established for the detection of adulteration. The work of Dr. Wiley in this field is summarized in his "Principles and Practice of Agricultural Analysis", which has passed through numerous editions. His interest in accurate analytical processes is also shown by his work in the organization and development of the Association of Official Agricultural Chemists, of which he was president in 1886, secretary from 1890 to 1912, and honorary president from 1912 until the present time. He is the only person who has attended every one of the forty-two annual meetings of this association and we are glad to have him with us again today. The work of the Association of Official Agricultural Chemists is intimately interwoven with that of the Bureau of Chemistry. The proceedings of its annual meetings from 1884 to 1913 and its methods of analyses until 1908 were published as Bulletins of the Bureau, whose chemists have always worked in close cooperation with their many State collaborators in the common effort to bring the official methods of analysis to the highest state of perfection. These methods are the basis of regulatory action in all parts of the United States and are a brilliant example of the fundamental importance of research to chemical control.

The investigations upon human foods which were conducted at the Bureau of Chemistry under Dr. Wiley's direction have been of a most varied character. There is only opportunity to refer very briefly to a few of those which have had the greatest bearing upon regulatory procedure. First in point of time is that famous collection of ten publications, prepared by such chemists as Bigelow, Crampton, McElroy, Richardson, Spencer, and Wiley, known as Bulletin 13 upon "Foods and Food Adulterants", beginning in 1887 and extending over a period of fifteen years. The series covered practically the whole range of manufactured foods and was the first comprehensive chemical survey of American food products ever to be undertaken. The work is unique of its kind, nothing of a similar nature having appeared either before or since.

Bulletin 13 was issued for the use and information of chemical experts and could not be appreciated by the public at large. But unless public opinion was aroused the campaign against food adulteration would gather no headway, so contemporaneous with the scientific series of Bulletin 13, the Bureau of Chemistry issued several popular bulletins upon the extent and character of food adulteration. These were prepared by Alexander J. Wedderburn and were based principally upon the splendid scientific work that had been conducted by chemists of the various State Boards of Health and Experiment Stations. The roll of State chemists and officers who took part in the passage of laws against adulteration is a long one, and their names deserve to be inscribed upon some tablet of honor as a permanent memorial. The list includes such valiant departed workers as Leach of Massachusetts, Johnson of Connecticut, Caldwell of New York, Frear of Pennsylvania, Weber of Ohio, Scoville of Kentucky, Ladd of North Dakota, Doolittle of Michigan, Fulmer of Washington, and many others too numerous to mention. This was an era of fine cooperation between State and Federal officials, all working zealously in the face of opposition for the attainment of one common end, which was reached on June 30, 1906, with the signing by President Roosevelt of the Federal Food and Drugs Act. This act represented the outgrowth of twenty years of extensive chemical investigations upon the food supplies of the Nation, and it will stand for all time as a splendid illustration of the important relationship of research to chemical control.

If there were time I should like to trace the relations of other lines of research conducted by scientists of the Department of Agriculture to other phases of Federal regulatory control, such for example as the research work of Drs. Haywood, McDonnell, and their co-workers upon insecticides to the passage of the Insecticides Act, of Dr. Veitch and his associates upon naval stores to the passage of the Naval Stores Act, and of the scientists of the Bureau of Animal Industry upon animal diseases to the Meat Inspection Act. The story is a long one, but it can all be summed up very briefly by the simple statement that without previous

fundamental research no satisfactory regulatory enactments can be passed and without constant accompanying research all regulatory enactments become lacking in efficiency. The latter part of this statement may perhaps be disputed by those who hold that, after research has shown the need and led to the passage of a control measure, the enforcement of the act resolves itself thereafter into the simple exercise of the machinery of law enforcement. This might be true if violators of the law were always content to continue in the same old way. But experience has shown that no one is more quick to apply the new discoveries of science to unlawful uses than those who adulterate the necessities of life. Scientists no sooner discover the virtues of radium, vitamins, and glandular extracts than the market is flooded with fictitious mineral waters, food products, and medicinal preparations purporting to give an over-credulous public the benefit of these new discoveries. There is an old saying that the adulterator of food and other products is always one step ahead of the control official. Remove control work from all contact with research and the adulterator will very speedily be not one, but one hundred steps in advance.

The various Federal regulatory laws such as the food and drugs act, the insecticides act and the naval stores act are not simply measures devised for the punishment of criminals. Their spirit is corrective, as well as punitive, and the scientists of the Bureau of Chemistry, as of other bureaus of the Department, are constantly engaged in assisting the manufacturers of food products, pharmaceuticals, insecticides, and naval stores to improve processes for increasing the purity of their products so that they will meet the requirements of the various Federal and State enactments. In this way the scientists of the Bureau of Chemistry have enabled the shippers of fruits; the canners of berries, sardines, and tomatoes; the manufacturers of foods and drugs; and the producers of insecticides, fungicides, turpentine, rosin, and many other commodities not only to bring their products within the requirements of regulatory laws but to effect great economies in the processes of preparation and manufacture. Constructive investigations of this character have meant the saving of millions of dollars to the agricultural industries of the United States. These trade problems are usually first brought to the attention of the research scientists of the Department by the field inspectors whose work is primarily of a regulatory character, and for the best performance of this advisory service there is necessarily a need of the closest contact between regulatory and research officials. This particular relationship of research to control, which has been so valuable and beneficial in its results, is one that is commonly overlooked.

The separation of research and regulatory work in agricultural chemistry has been the general practice in all European countries where it was recognized even fifty years ago that these two lines of activity were

so different in purpose and method that their complete segregation under different administrative offices, with different laboratories and personnel, was effected at an early date. The combination of research and control work adopted by most of the State Agricultural Experiment Stations of the United States at the time of their establishment, was regarded by European scientists as a mistake and there has been a growing realization of this in America during the past twenty-five years. On account of the interference of the regulatory work with the research activities of the stations, many of the latter have either transferred all their law enforcement work to State officials or have established independent laboratories where the two lines of activity can be conducted without mutual disturbance. A few experiment station directors are still of the opinion that some connection of the stations with regulatory work upon fertilizers, feeding stuffs, insecticides, and human foods is advisable not only for the advantage of the agricultural contacts but because of the unfortunate degeneration of the regulatory work in some States where it lost the benefit of the research atmosphere and came under the control of corrupting political influences.

The research chemists of the State experiment stations and Federal bureaus have in many cases been overwhelmed by the demands of regulatory work and their scientific investigations have in consequence been badly hampered. These two lines of activity are in many respects mutually repellent and require the attention of officials of very different qualifications. The control chemist, because of the detriment of delayed decisions to industry and commerce, is obliged to form his conclusions quickly, even though in many cases these conclusions may be wrong. The research chemist, on the other hand, must form his conclusions with more deliberation because of the necessity of verifying his work by all the possible checks at his disposal. The regulatory chemist, owing to the demands of law enforcement, limits his attention to the small five per cent, or so, of products which constitute infractions of the various State and Federal regulatory enactments; the research chemist, on the other hand, is more interested in the larger ninety-five per cent, or so, of products which meet the requirements of those laws.

Another factor which has tended towards a segregation of research and regulatory work is the growing development of extreme types of specialists or experts in each of these fields. The day of the versatile all-sided food chemist of thirty years ago has passed. The technic of mold counts, hydrogen-ion values, vitamin potency, and many other determinations cannot be mastered by the simple reading of analytical directions, but requires weeks and months of careful specialized training. The effect of specialization has always been towards a constant repartitioning of a field into smaller and smaller units, just as the old land grants of Colonial times have been progressively subdivided into

States, counties, cities, boroughs, and wards. The accumulations of court decisions and other regulatory data during the past twenty years are now so voluminous that the control official must devote much of his time to a study of legal procedures and precedents. The research chemist would regard such an expenditure of his time as wasted effort, and our experiment station scientists are not to be blamed for wishing to be relieved of the growing intrusion of regulatory demands upon their research activities.

A separation of research and control work under different administration offices must be recognized as inevitable. Care must be taken, however, in making this separation that certain advantages of the former combination be not lost. The immense value and importance of research to control has already been pointed out. Regulatory work was born of research and under the parental care of research it has grown to full maturity. In this period of close contact at the experiment stations and Government bureaus the research and regulatory work became mutually helpful. By its suggestion of practical subjects for investigation the regulatory work acted as a valuable guide in preventing research from going astray, as it so often does, into fields that were visionary or lacking in utility. The correcting influence of research in return operated to make the work of law enforcement more just, more reliable and more effective.

There was also developed as a result of this contact a valuable type of scientist who shared equally the regulatory and research points of view, men of broad outlook and vision, of whom Dr. Hassall in England and Dr. Beck in the United States were the original prototypes and of whom the later leaders in the crusade against food adulteration were the worthy successors. Such men have been of the greatest service in matters pertaining to law enforcement and it will be a most serious loss to the future efficiency of control work if the development of officials of this type is in any way curtailed.

A teacher of agricultural chemistry at one of the State colleges once made the remark that the training of students in the methods and purposes of chemical control had suffered a serious loss at his institution because the opportunity of studying such work by direct observation was no longer possible after the regulatory work had been transferred elsewhere. He admitted, however, that the advantages of segregating the research and control work outweighed the disadvantages, and this seems to be the general verdict of those who have most carefully considered the question. There must, however, always be at our State colleges and universities teachers who have had sufficient experience in regulatory work to give competent instruction in this field. If this be done and the administrators of law enforcement keep their control work abreast with the latest developments of research, we can look forward

with confidence to the future of this most important branch of public service.

Finally, I would emphasize the great advantage which is derived from the occasional meeting together in conference of research and regulatory officials—such meetings, for example, as those of our Association of Official Agricultural Chemists, where the mutual relations of research and control are discussed in all their various bearings. As mentioned in my address of last year “the members of our association that are engaged in these separate fields are enabled at our meetings to give one another the benefit of mutual assistance and advice”. The continuance of this happy relationship is the best assurance of future progress in both of these branches of agricultural chemistry.

MALTED MILK.

A STUDY OF THE ACTION OF THE ENZYMES OF MALT UPON MILK SOLIDS DURING MANUFACTURE.

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INTRODUCTION.

“Malted milk² is the product made by combining whole milk with the liquid separated from a mash of ground barley malt and wheat flour, with or without the addition of sodium chloride, sodium bicarbonate, and potassium bicarbonate, in such a manner as to secure the full enzymic action of the malt extract, and by removing water. The resulting product contains not less than seven and one-half per cent (7.5%) of butterfat and not more than three and one-half per cent (3.5%) of moisture”.

The production involves (1) the preparation of a “malt-flour” infusion by the process of mashing, and (2) the evaporation to dryness of the “infusion-milk” mixture. For a detailed description of the process the reader is referred to Hunziker³.

The changes that take place in the malt and flour ingredients during the mashing process are chiefly biological and are well understood. The changes that take place in the second step, however, the evaporation of the “infusion-milk” mixture, have not, to the knowledge of the writers, been investigated. It was for the purpose of determining whether biological activities occur in this step that the study recorded in this paper was undertaken. Moreover, it seemed necessary to establish definitely

¹ The greater part of the laboratory work upon which this publication is based was conducted in the Food Control Laboratory while that laboratory was a part of the Bureau of Chemistry and under the direction of Dr. F. C. Blanck. The authors take pleasure in expressing their appreciation to the members of the trade for their hearty cooperation.

² Definitions and Standards for Food Products, F. D. No. 2, p. 4, issued December, 1928.

³ Condensed Milk and Milk Powder, 4th ed., pp. 531-48.

the identity of malted milk, because a tendency to depart from the original and accepted method of manufacture has been noted since the expiration of the patent¹, and products of similar composition but of different physical characteristics have been offered in the market under the name of malted milk.

According to statistics issued by the Bureau of Agricultural Economics, United States Department of Agriculture, the production of malted milk in the United States in 1926 reached a total of approximately 20,500,000 pounds, containing the equivalent of about 4,500,000 gallons of liquid milk.

The minimum quantity of milk fat in malted milk is fixed by the existing standard; there is, however, no provision in it relative to the ratio of the flour to the malt. Calculated on a basis of 7.5 per cent butter fat, the composition of standard malted milk is approximately 27 per cent milk solids and 73 per cent malt-flour solids. This calculation does not take into consideration the moisture content nor the small quantities of sodium chloride and alkali carbonates used in the process of manufacture.

It is obvious that the chemical composition of a malted milk prepared under the existing standard will vary somewhat in the quantity and quality of its main constituents—butter fat, proteins and carbohydrates. These variations, however, will not affect the identity of malted milk, since they are due to different factory practices. The average butter fat, protein, carbohydrate and ash content of market malted milks is about 8, 15, 72, and 4 per cent, respectively. They do not contain starch.

The effects of evaporation upon the malt and milk components are evident in some of the well-defined characteristics of malted milk. It has an agreeable malt odor and flavor, and despite its relatively high milk-solids content it is free from a tallowy taste. Microscopically it presents a picture that cannot be mistaken for any product of similar nature. The malt extract and the milk solids are incorporated into homogeneous masses having a stippled appearance. When mixed with water these masses produce a suspension which does not break up even after long standing, another characteristic difference between malted milk and the products prepared by mixing dry whole milk and dry malt extract. The keeping qualities of malted milk have been explained by the fact that the fat globules are protected against the deteriorating action of air by a coating or envelope of gluten, sugars and salts. Here again is evidence of the intimate relation of the malt-flour infusion with the milk solids that is directly attributable to the influence of the evaporating process. From the standpoint of chemical and physical characteristics, therefore, it is evident that the identity of malted milk is

¹ Granulated Food for Infants, Patent No. 278967, June 5, 1883.

well established, and it only remains to determine to what extent milk solids are changed during the evaporation process as a result of biological influences.

It is not the intention of the writers to enter into a discussion of the complex activities of the many enzymes that are contained in a properly prepared malt. The literature contains numerous references regarding the nature of these enzymes, the most favorable conditions (pH and temperature) for their digestive influences, and the methods for measuring the capacity of their activities. It is also well known that when properly prepared, malt contains a number of ferments which, under optimum conditions, break down the complex starch, protein and fat molecules into simpler substances of comparatively low molecular weight. In fact, the changes in chemical composition that take place in the manufacturing process, from the steeping of the barley in the malting operations to and including the preparation of the malt-flour infusion, are almost wholly the result of enzymic activities.

It is not to be expected that the same functions that have been ascribed to these enzymes in homogeneous substrates will manifest themselves in a heterogeneous substrate as found in milk, although the general conditions of temperature and pH optima for proteolytic activities that have been found in these investigations agree well with those recorded in the literature.

Since the malt-flour infusion is the active agent for any digestion which may manifest itself in the evaporation of the malt infusion-milk mixture, the characteristics of malt infusions prepared under varying conditions of temperature were studied.

THE MALT-FLOUR INFUSION.

Only the general methods practiced in the factory for the preparation of the malt-flour infusion are available. Ground malt and wheat flour in about equal proportions are mashed to produce an infusion containing about 15 per cent of solids. The mash is gradually raised to the saccharifying temperature and held there until conversion of starch has been effected. It is known that this temperature is about 150°F., but information regarding the manner of maintaining it during mashing is not available. That the time and manner of raising the infusion to the saccharifying temperature will affect its dextrin and sugar content, however, is shown in the data obtained in the examination of 3 samples of infusions collected at malted milk factories. Three samples were obtained from the tanks used for collecting the infusions, and they were sterilized in the bottle in boiling water. Samples No. 1 and No. 2 contained a heavy deposit, while Sample No. 3 was practically clear. The analyses were made on the unfiltered samples. The results are shown in Table 1.

TABLE 1.

Chemical composition of malt-flour mashes (infusions) obtained from three malted milk factories.

SAMPLE NUMBER	SOLIDS IN VACUO	NON-SUGAR SOLIDS	SUGARS— DIRECT AS MAL- TOSE + 1 H ₂ O	POLARIZATION NORMAL WEIGHT 200 MM. TUBE	SUGARS IN SOLIDS	ETHER EXTRACT	ACIDITY (AS LACTIC ACID)	PROTEIN (N × 6.38)	ASH	P ₂ O ₅	CRUDE FIBER
	per cent	per cent	per cent	V°.	per cent	per cent	per cent	per cent	per cent	mg. per 100 grams	per cent
1	18.70	5.86	12.84	30.7	69	0.36	0.26	0.73	0.24	72.5	0.03
2	15.57	5.01	10.56	25.1	68	0.39	0.49	1.90*	0.18	47.0	0.04
3	16.08	2.47	13.61	26.2	84	0.05	0.20	0.80	0.23	50.0	0.003

* Average of six determinations.

The composition of Sample No. 3 is strikingly different from that of the other two samples. Judging from its general composition, a cleaner separation of the insoluble material of the mash was obtained in its preparation. This is evident in the low percentages of crude fiber, ether extract and non-sugar solids. The high sugar in solids of this sample denotes a greater diastatic digestion and, presumably, a lower dextrin content. A comparative analysis of the data also shows that the mashing processes followed in the three factories varied somewhat and that the differences in chemical composition are directly attributable to enzymic actions produced by the different temperatures used during the mashing operations.

Realizing the importance of temperature in its effect upon the composition of the malt infusion, experiments were conducted for the purpose of establishing the best conditions for obtaining maximum extraction of solids from the malt-flour mixture.

INFLUENCE OF TEMPERATURE ON THE CONVERSION OF STARCH IN THE MASHING PROCESS.

As a basis for these experiments, malt-flour infusions were prepared in much the same way as in factory practice.

To 50 grams of a good grade of hard wheat flour in a copper mashing beaker 1.5 grams of sodium chloride, 5 cc. of 25 per cent solution of thymol in alcohol, and 100 cc. of tap water were added, and the contents were stirred into a smooth paste. Thymol was added to guard against bacterial development. Although smaller quantities were tried, it was found that 5 cc. is required to inhibit bacterial action within 4 hours. Even with this strength of thymol it was found that bacterial activities occurred when longer periods of digestion at low temperatures were

used. To the paste 200 cc. of boiling water was added, and the temperature was adjusted to 70°C. and held for 15 minutes; 50 grams of ground malt of high diastatic quality was added after its temperature had been adjusted to assure the desired temperature for the experiment. The mixtures were held at that temperature for the period of time indicated in Table 2. Then the contents of the beaker were made to 406 grams with tap water and immediately centrifuged at high speed. The liquid portion obtained is termed "malt infusion" in these experiments. Although this method of preparing the infusion is not strictly in accord with factory practices, it is believed that it answers the purpose of the study.

The mashes were tested for conversion of starch by the iodine test. For the pH measurements a modification of the Bunker type vessel, as suggested by Waterman¹, was used. The calomel electrode was of the standard type. The measurements were made on a type "K" Leeds and Northrup potentiometer. This set-up permitted of measurements within the narrow limits of 1/100 pH unit. The results are shown in Table 2.

TABLE 2.

Characteristics of malt infusions prepared at temperatures of 30°-70°C.

MASHING TEMPERATURE	MASHING PERIOD	CONVERSION	SOLIDS	NON-SUGAR SOLIDS	SUGARS— AS MALTULOSE + 1 H ₂ O	SUGAR IN SOLIDS	pH		ACIDITY (AS LACTIC ACID)	
							Initial	After 16 hours	Initial	After 16 hours
°C.	hours		per cent	per cent	per cent	per cent			per cent	per cent
30	4	Incomplete	13.55	3.86	9.69	71.5	5.90	5.91	0.16	0.16
35	4	Incomplete	13.45	3.28	10.17	75.6	5.85	5.94	0.15	0.16
45	4	Incomplete	14.40	2.86	11.54	80.1	5.89	5.88*	0.17	0.17†
55	4	Incomplete	15.40	3.11	12.29	79.8	5.80	5.81	0.18	0.18
65	1½	Complete after 1 hour	18.70	4.40	14.30	76.4	5.72	‡	0.14	0.14¶
70	1½	Complete after ¾ hour	19.30	5.15	14.15	73.3	5.71	5.68§	0.13	0.13

* After 40 hours, 5.83.

† After 40 hours, 0.20.

‡ After 4 hours, 5.71.

¶ After 4 hours, 0.14.

§ After 4 hours, 5.68.

|| After 4 hours, 0.13.

Obviously no manufacturer would adopt the lower temperatures in his process because of loss of solids. It is, of course, quite possible to complete the conversion by raising the temperature finally to 65°C. and holding at that temperature for 1 hour.

¹ *This Journal*, 1927, 10: 390.

The pertinent facts developed by the experiments are (1) that the mashing temperature does not materially affect the hydrogen-ion concentration; (2) that the highest sugar degree (percentage of sugar in solids) is obtained at a temperature of 45°C.; (3) that for complete conversion of starch a temperature of 65°C. is necessary; and (4) that a malt infusion prepared at 65°C. has a pH of about 5.7 and an acidity in terms of lactic acid of approximately 0.14 per cent.

Having established the characteristics of the malt infusion, the writers proceeded to determine whether biological changes occur in the evaporation process. This phase of the work was restricted to the study of the effect of the more important enzymes, lipase and protease. Before the changes occurring under factory conditions were considered, however, it seemed desirable to ascertain under what conditions these enzymes exert their greatest influence.

LIPOLYTIC ACTIVITIES.

The enzyme lipase, when permitted to act upon butterfat, splits off acids, thereby liberating glycerin. While this action is usually recognizable by an increase in acid when the substrate and ferment are in a state of homogeneity, it is obvious that acidity measurements in products representing mixed activities may not be conclusive. Although it is recognized that no definite conclusion should be reached with respect to the action of lipase before lipase of malt has been isolated and its action upon milk fat in the absence of interfering substances has been tested, it did not seem advantageous to the object of this investigation to attempt such work.

For determining lipolytic activities, malt infusions were prepared at temperatures ranging from 30°–65°C., with and without the addition of salt. To these infusions (1) neutral butterfat and (2) powdered whole milk were added and incubated at the temperatures used for the preparation of the mashes. Since there is some doubt as to the solubility of lipase in the infusions, similar experiments were also conducted by permitting the entire mash (salt omitted), *not centrifuged*, to act on butterfat.

In the experiments powdered whole milk was used as a substitute for fluid milk. The butterfat was prepared by melting a good grade of sweet market butter, filtering while hot to remove curd, washing with distilled water until neutral, and finally washing with water containing thymol. The powdered whole milk used was a good grade obtained in the market. The mash was prepared as described previously in this paper.

For recognizing lipolytic activities, acidity measurements were made in terms of 0.1 *N* acid, phenolphthalein being used as the indicator.

TABLE 3.

Effect of the malt infusion on neutral butterfat.

400 cc. of malt infusion (no salt added). 10 grams of butterfat, pH unadjusted.

INCUBATION PERIOD	30°C.		45°C.		55°C.		65°C.	
	ACIDITY*	pH	ACIDITY*	pH	ACIDITY*	pH	ACIDITY*	pH
hours								
0	1.00	5.76	1.20	5.75	1.40	5.67	1.50	5.75
1	0.90	5.79	1.20	5.76	1.40	5.66	1.50	5.75
2	0.96	5.76	1.20	5.74	1.40	5.65	1.50	5.70
3	1.00	5.75	1.20	5.76	1.40	5.67	1.50	5.70
4	1.00	5.76	1.20	5.75	1.40	5.67	1.50	5.71
20	1.60	4.81	4.00	1.40	5.93†	1.50	5.64

* 0.1 N acid per 10 cc.

† It is recognized that this result is not in correlation with the other results.

The results obtained by using infusions in which salt was added and also those obtained when the entire mash was used were practically the same with respect to acidity changes as those recorded in Table 3. The results on powdered whole milk are presented in Table 4.

TABLE 4.

Effect of the malt infusion on powdered whole milk.

250 cc. of malt infusion (salt added). 19 grams of powdered whole milk.

INCUBATION PERIOD	30°C.		45°C.		55°C.		65°C.	
	ACIDITY*	pH	ACIDITY*	pH	ACIDITY*	pH	ACIDITY*	pH
hours								
pH UNADJUSTED.								
0	1.65	6.07	1.85	6.10	1.90	6.05	1.65	6.04
1	1.65	6.08	1.85	6.07	1.90	6.05	1.65	5.99
2	1.65	6.10	1.85	6.07	1.90	6.04	1.65	5.94
3	1.65	6.10	1.75	6.10	1.85	6.01	1.65	5.96
4	1.65	6.10	1.80	6.08	1.90	6.01	1.65	5.92
20	7.50	4.30	10.55	3.70	1.90	5.99	1.95	5.87
pH ADJUSTED TO 5.0.								
0	3.40	5.05	3.90	5.06	3.85	5.06	3.55	5.04
1	3.35	5.07	3.70	5.02	3.65	5.04	3.35	5.07
2	3.38	5.05	3.65	4.99	3.75	5.06	3.35	5.06
3	3.42	5.07	3.75	4.94	3.85	5.00	3.45	5.05
4	3.40	5.00	3.75	4.94	3.85	5.03	3.50	5.00
20	7.15	4.00	7.60	4.01	6.05	4.42	3.50	5.02
pH ADJUSTED TO 6.5.								
0	0.93	6.66	1.20	6.54	1.20	6.51	1.20	6.51
1	0.95	6.65	1.20	6.47	1.20	6.45	1.20	6.42
2	0.92	6.64	1.20	6.43	1.20	6.45	1.20	6.42
3	0.92	6.61	1.10	6.44	1.20	6.44	1.20	6.38
4	0.95	6.58	1.15	6.46	1.30	6.44	1.20	6.37
20	5.30	4.56	4.80	4.33	3.00	5.53	3.60	5.17

* 0.1 N acid per 10 cc.

The data presented in Tables 3 and 4 show no definite increase in acidity through enzymic activity. After 20 hours' incubation there is an increase of acid over the quantity shown in the original material, which is not due to enzymic activity. In almost all the instances where there was a pronounced increase of acid, the incubation mixtures had the offensive odor characteristic of bacterial decomposition. This odor was particularly noticeable in the mixtures incubated at the lower temperatures, 30°–45°C., which is the optimum range for certain types of bacteria. One of the mixtures in which the decomposition was most pronounced was submitted to microbiological examination and shown to be alive with bacteria of various types.

From the results shown in Tables 3 and 4 it is concluded that under the conditions of the experiments no lipolytic action takes place when malt infusions prepared at temperatures of 30°–65°C. are permitted to act on butterfat or powdered whole milk. Since these temperatures and pH ranges cover the conditions that may occur in factory practice it is very doubtful that digestion of fat occurs during the manufacturing process. This conclusion is, of course, open to the criticism that the experiments do not take into consideration the concentration of the enzyme through the progressive removal of the water, under which circumstance increased activity is possible. However, it is believed that the negative results obtained justify this conclusion.

PROTEOLYTIC ACTIVITIES.

The literature contains numerous references to the action of enzymes on proteins and also describes methods for determining to what extent they affect digestion. Generally the methods are applicable only to more or less pure materials. Although it is evident that if the materials are heterogeneous, measurements such as viscosity and acidity cannot be used to demonstrate the activity of a single enzyme, for the sake of completeness these determinations were included in the experiments. It was believed that for measuring proteolytic digestion the increase in soluble proteins and amino acids would serve as the best criterion. The methods used for determining the changes occurring during digestion were the following:

1. *Amino nitrogen.* A large unit of the Van Slyke apparatus was used.
2. *Soluble proteins.* The soluble proteins were determined on filtered portions of the digestion mixtures by the modified Kjeldahl-Gunning-Arnold method. Copper sulfate was used as the catalyst.
3. *Viscosity measurements.* For the determination of viscosity relative values were considered satisfactory. In the table this relation is indicated as $\frac{\text{seconds for sample}}{\text{seconds for water}}$ at 35°C. Briefly the apparatus consisted of a 50 cc. pipet having a capillary outflow of 24.2 seconds at 35°C. for distilled water.

4. *pH measurements.* Made with the modified bunker type electrode vessel previously described.

5. *Acidity.* Measured in terms of 0.1 *N* hydrochloric acid, phenolphthalein being used as indicator.

The studies of proteolytic digestion were conducted in much the same manner as those in the lipolytic experiments. It was found impracticable to use liquid whole milk. Difficulty was also experienced when powdered whole milk was used as the substrate, because it was found impossible to obtain the necessary clear filtrates. After unsuccessful attempts to use powdered whole milk, milk proteins precipitated from powdered whole milk with 70 per cent alcohol were used. Additional experiments were also made with Hammerstein casein as the substrate.

TABLE 5.

Effect of the malt infusion on milk protein.

250 cc. of malt infusion; 5 grams of milk protein.

INCUBATION TEMPERATURE— °C.	pH UNADJUSTED			pH ADJUSTED TO 5.00			pH ADJUSTED TO 6.50		
	INCUBATION PERIOD— HOURS			INCUBATION PERIOD— HOURS			INCUBATION PERIOD— HOURS		
	0	4	20	0	4	20	0	4	20
AMINO NITROGEN, MG. PER 100 CC.									
35	33.6	42.0	45.4	32.3	39.0	47.8	36.1	43.6	46.0
45	41.9	51.1	52.0	40.1	47.0	58.3	37.5	42.8	48.7
55	41.2	46.4	48.9	40.4	47.6	54.8	41.3	47.8	54.1
65	36.1	42.5	44.0	33.8	39.4	38.8	32.1	37.3	34.8
TOTAL NITROGEN, MG. PER 100 CC.									
35	159	162	166	128	152	171	151	164	177
45	174	183	191	166	177	189	165	183	184
55	197	201	201	184	198	205	192	208	212
65	...	181	187	159	164	165	164	169	184
VISCOSITY $\frac{\text{SECONDS FOR SAMPLE}}{\text{SECONDS FOR WATER}}$ 35°C.									
35	1.314	1.260	1.169	1.326	1.252	1.180	1.322	1.269	1.190
45	1.314	1.216	1.182	1.314	1.218	1.161	1.322	1.248	1.206
55	1.399	1.281	1.231	1.429	1.310	1.252	1.380	1.305	1.235
65	1.603	1.454	1.355	1.669	1.533	1.413	1.595	1.479	1.429
ACIDITY—CC. 0.1 <i>N</i> ACID PER 100 CC.									
35	13.0	14.0	26.5	19.0	20.5	24.0	7.5	9.5	10.5
45	14.5	16.0	16.0	21.0	22.5	25.0	9.0	10.0	11.0
55	13.0	16.0	16.0	21.0	22.0	25.0	10.0	12.0	13.0
65	14.0	14.0	14.0	20.0	18.5	20.0	6.0	9.0	9.5
pH.									
35	5.78	5.72	4.96	5.07	4.99	6.47	6.05	6.35
45	5.73	5.61	5.75	4.95	5.06	5.07	6.41	6.03	6.04
55	5.75	5.71	5.67	4.93	5.01	5.07	6.51	6.17	6.23
65	5.77	5.62	5.61	4.94	5.04	5.07	6.58	6.38	6.29

The mashes were prepared as described previously. To a 250 cc. portion of the mash, 4 grams of casein or 5 grams of precipitated milk protein was added. After being thoroughly beaten, the mixture was allowed to stand 15 minutes. The quantity of substrate used was greatly in excess of the quantity soluble in the infusions. After standing 15 minutes a portion of the mixture was immediately filtered, and the filtrate was examined. The remaining portion of the mixture was incubated under the conditions indicated in the tables. The substrates casein (Hammerstein) and milk protein contained 0.045 and 0.057 per cent amino nitrogen, respectively. If it be assumed that the quantities of the substrate used are completely soluble in the infusion, then the amino nitrogen introduced from these sources would be 0.7 and 1.1 mg. in 100 cc. of incubation mixture, respectively.

The digestions were conducted at the carefully controlled temperature and pH ranges indicated in the tables. The data obtained in the experiments when milk proteins were used as the substrate are given in Table 5.

The results in Table 5 show an increase in amino nitrogen and soluble protein at all temperatures and pH ranges after 4 hours and after 20 hours of incubation. The greatest increase in amino nitrogen occurs at 45°C. at a pH of 5.0 after 20 hours. The viscosity changes are considerable, showing sharp decreases with the length of the incubation period. These changes are not due wholly to protein digestion; rather they are due to conversion of dextrans. The acidities show a slight increase in all cases after 4 hours of incubation.

The results obtained when casein was used as the substrate are given in Table 6.

In Table 6, as in the previous tables, the greatest digestion occurs at a temperature of approximately 45°C. What has been said about viscosity and acidity changes also applies to these results.

Thus far no account has been taken of the change in amino nitrogen occurring during incubation in the malt infusion itself. Since it has been shown that the greatest digestion takes place at a pH of 5.0 an examination of the malt infusion so adjusted and at temperatures of 35°-65°C. was made. The results are given in Table 7.

The greatest increase in amino nitrogen takes place at 35°C.; from this temperature the decrease is gradual until at 55° and 65°C. no change is recorded.

The amino nitrogen results at a pH of 5 on milk protein and casein, taken from Tables 5 and 6, are also given in Table 7, and the net increase due exclusively to digestion of milk protein and casein has been computed.

If the assumption that an increase in amino nitrogen indicates protein digestion is correct, then the data in Table 7 leave no doubt that pro-

TABLE 6.

Effect of the malt infusion on casein.

250 cc. of malt infusion; 4 grams of casein; pH approximately 5.

INCUBATION TEMPERATURE— °C.	INCUBATION PERIOD—HOURS			INCREASE DECREASE (—)	
	0	4	20	4	20
AMINO NITROGEN—MG. PER 100 CC.					
35	35.1	37.3	55.2	2.2	20.1
45	33.0	46.9	65.9	13.9	32.9
55	39.2	44.7	60.8	5.5	21.6
65	31.6	34.5	40.9	2.9	9.3
TOTAL NITROGEN—MG. PER 100 CC.					
35	140	158	177	18	37
45	166	190	216	24	50
55	165	199	223	34	58
65	150	158	165	8	15
VISCOSITY $\frac{\text{SECONDS FOR SAMPLE}}{\text{SECONDS FOR WATER}}$ 35°C.					
35	1.310	1.219	1.169	—0.091	—0.141
45	1.293	1.223	1.182	—0.070	—0.111
55	1.417	1.310	1.265	—0.107	—0.152
65	1.608	1.479	1.392	—0.129	—0.216
ACIDITY CC. 0.1 N NaOH PER 100 CC.					
35	12	15	17	3	5
45	15	18	20.5	3	5.5
55	15	17	21	2	6
65	13	13.5	15	0.5	2
pH.					
35	5.22	5.21	5.16	—0.01	—0.06
45	5.23	5.14	5.09	—0.09	—0.14
55	5.06	5.05	—0.01
65	5.10	5.06	5.01	—0.04	—0.09

teolytic activity occurs when a properly prepared malt infusion is permitted to react with milk proteins.

The work thus far recorded on proteolytic activities relates to determinations made under controlled conditions. It still remains to be shown whether or not such activities occur under factory conditions. The pH of a mixture of malt infusion and milk is in the neighborhood of 6.00. The final mashing temperature employed in the factory is about 65°C. In Table 5 it will be seen that during an incubation period of 4 hours at a pH of 6.50 and a temperature of 65°C. less pronounced proteolytic digestion occurs than under all the other conditions. A temperature of 65°C. is considered sufficiently high to cause marked inactivation of proteolytic enzymes of the malt. However, it should be remembered that in the manufacture of malted milk the high temperature of 65°C. is maintained only during the mashing process. The temperature dur-

TABLE 7.

Net increase of amino nitrogen due to digestion of milk protein and pure casein by malt infusion.

AMINO NITROGEN MG. PER 100 CC.	35°C.			45°C.			55°C.			65°C.		
	INCUBATION HOURS			INCUBATION HOURS			INCUBATION HOURS			INCUBATION HOURS		
	0	4	20	0	4	20	0	4	20	0	4	20
Infusion milk-protein mixture.....	32.3	39.0	47.8	40.1	47.0	58.3	40.4	47.6	54.8	33.8	39.4	38.8
Malt infusion.....	31.4	35.9	39.4	34.6	37.9	39.5	39.3	39.2	39.4	33.8	33.8	33.8
Milk protein—net increase.....	...	3.1	8.4	...	9.1	18.8	...	8.4	15.4	...	5.6	5.0
Infusion casein mixture.....	35.1	37.3	55.2	33.0	46.9	65.9	39.2	44.7	60.8	31.6	34.5	40.9
Malt infusion.....	31.4	35.9	39.4	34.6	37.9	39.5	39.3	39.2	39.4	33.8	33.8	33.8
Casein—net increase	1.4	15.8	...	9.0	26.4	...	5.5	21.4	...	0.7	7.1

ing the evaporation in vacuo (27 inches mercury) is considerably lower than 65°C., approaching the optimum for protein digestion, and it may, therefore, be quite possible that factory conditions are more conducive to digestion than the conditions maintained in the experiments—that is, 65°C. throughout the incubation period.

In the hope that it might be possible to duplicate in the laboratory the conditions obtaining in factory practice the Claissen flask was substituted for the vacuum pan. Although it is obvious that the evaporation cannot be carried to dryness in the Claissen flask, this apparatus provides a means for conducting the evaporation to the point where, in the factory, the mixture is ready for the finishing pans.

Two batches of malt infusion, made in the manner previously described, were prepared at a temperature of 65°C. To one of the batches sufficient fluid whole milk was added to yield 7.5 per cent butterfat on the dry basis and to the other water was added in place of the milk; 400 cc. of each of these mixtures was evaporated in Claissen flasks under a vacuum of 27 inches of mercury. After the evaporation had proceeded to about one-third the original volume (3 hours) the contents of the flasks were made to original weight with water. The acidity, amino nitrogen and pH values were immediately determined on the reconstructed batches and again after 16 hours of incubation at 55°C., which is the approximate temperature in the vacuum kettle.

The results in Table 8 show that there is practically no proteolytic digestion in the 3 hour evaporation period, but a very decided increase is indicated after 16 hours' incubation at 55°C.

TABLE 8.

Changes occurring in A—malt infusion plus milk—and B—malt infusion plus water—during 3 hours of evaporation in a vacuum of 27 inches mercury and subsequent incubation.

	SAMPLE A			SAMPLE B		
	pH	Amino nitrogen	Acidity	pH	Amino nitrogen	Acidity
		<i>mg. per 100 cc.</i>	<i>cc. 0.1 N HCl per 100 cc.</i>		<i>mg. per 100 cc.</i>	<i>cc. 0.1 N HCl per 100 cc.</i>
Original.....	6.75	38.1	8.0	6.49	31.7	8.0
After 3 hours' evaporation (residue to original weight).....	6.58	40.1	8.0	6.43	34.5	8.0
After 16 hours' incubation at 55°C.....	6.54	48.1	9.0	6.45	39.2	9.0

An additional experiment, for which a similar batch of malt was used and the same conditions of temperature and vacuum were maintained, was made, but instead of 3 hours the time of action was prolonged to 7 hours by intermittently evaporating and resting. The results obtained were practically the same as in the previous experiment.

From the data obtained it is evident that no proteolytic digestion occurs during the first 3 hours of evaporation. Whether there is an accelerated action of proteolysis during the final evaporation to dryness, through the concentration of the enzyme, is not demonstrable in the laboratory.

Before starting this investigation, one of the writers inspected a number of malted milk factories. At each of two of the factories visited two batches of malted milk were prepared: one represented the regular factory run; the other had the same ingredients, but in its preparation the enzymes were destroyed by heating the malt-infusion-milk mixture to boiling before it was subjected to evaporation. These products were finally dried in finishing pans. For determining the proteolytic activity of the four products with casein as the substrate the following procedure was used:

Five grams of casein (Hammerstein) was beaten in 50 cc. of tap water to a fine suspension and allowed to stand with occasional shaking for 30 minutes at room temperature. To this mixture was added 25 grams of the material suspended in 250 cc. of tap water. In order to inhibit bacterial action 10 cc. of a 25 per cent alcoholic thymol solution was added. The mixture was adjusted to a pH of approximately 5.0 by the addition of normal hydrochloric acid and incubated at 45° and 55°C. This procedure was followed on each of the four products. The results are shown in Table 9.

TABLE 9.
Effect of malted milk and inactivated malted milk on casein.
 A—True malted milk. B—Inactivated malted milk.

SAMPLE NO.	TEMPERATURE	A			B		
		AMINO NITROGEN—MG. PER 100 CC.			AMINO NITROGEN—MG. PER 100 CC.		
		INCUBATION PERIOD—HOURS			INCUBATION PERIOD—HOURS		
		0	4	20	0	4	20
	°C.						
1	45	15.6	17.7	18.1	17.8	19.9	18.2
1	55	16.9	21.1	21.0	21.4	22.4	22.7
2	45	17.7	17.7	18.1	24.5	22.1	21.6
2	55	21.4	24.5	23.1	23.7	23.6	21.6

Apparently the conditions obtaining in the factory do not completely destroy the protein digestive properties of the malt infusion. While under the conditions of the experiments (dilution 1 to 12) the digestion is small, it may be assumed that with a greater concentration of the enzyme the results would have been more convincing.

DIASTATIC ACTIVITIES.

Of the numerous enzymes contained in malt, diastase, under optimum conditions, shows the greatest digestive activities. According to the literature the enzyme is destroyed at a temperature of 80°C., which is considerably above the highest temperature (65°C.) occurring in the manufacture of malted milk. If the high resistance of diastase to heat is considered, it might be supposed that malted milk would be diastatically active, but experiments with a number of the better known brands of malted milk, wherein soluble starch was used as the substrate, showed only slight digestion when tested with iodine. Measurements of pH of three of the better known brands of malted milk, which had been stored in the laboratory for two years, were found to be about 7.2. Failure of malted milk to convert starch is very likely due to this high pH. In this investigation it was found that maximum starch conversion takes place at a pH of about 5.9 and at a temperature of 45°C.

The three stored malted milks mentioned above, adjusted to a pH of about 5.9, were permitted to act upon soluble starch for 1 hour at a temperature of 45°C.; 100 grams of the incubation mixture contained 3 grams of starch and 5 grams of malted milk. The diastatic activity was measured by the increase in sugar, expressed as maltose. The results, given in Table 10, show that even after long storage at room temperature malted milk has distinct digestive properties.

TABLE 10.

Diastatic activities of stored malted milks.

SAMPLE NUMBER	MALTOSE 1 H ₂ O BEFORE DIGESTION	MALTOSE 1 H ₂ O AFTER DIGESTION	INCREASE IN SUGAR
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	2.18	2.48	13.8
2	3.08	3.24	5.2
3	2.76	2.99	8.3

It has been claimed that the action of enzymes in the process of manufacture improves the keeping qualities of malted milk. Organoleptic observations made on malted milks prepared in the regular way and on products obtained by drying inactivated malt-infusion-milk mixtures do not confirm this conclusion, since it was found that both products kept equally well after two years' storing at room temperature.

Before final discussion of the identity of malted milk a few general remarks regarding phases of the manufacture not previously considered may be made.

In factory practice alkali carbonates are added during the evaporation, principally for the purpose of suppressing the curdling of casein, which occurs at a pH of about 4.8. Since in this investigation it has been shown that the pH of commercial malted milks lies in the neighborhood of 7.2, it would seem that the quantity of "neutralizer" used by the manufacturer is greatly in excess of that required to prevent the curdling of casein. In fact, in the digestion experiments in which the pH of malted milk was dropped from 7.2 to 5.9 by the addition of acid, no curdling was noticeable.

The original patent for preparing malted milk specifies evaporation in the so-called finishing pan, which is a small vacuum kettle equipped with a mechanical agitator. The partially evaporated malt-milk mixture is run into this kettle and gradually reduced to dryness under high vacuum (27 inches mercury). To prevent local overheating, the mass is kept in motion by the agitator. Toward the end of the drying period the material pulls very much like molasses taffy; it also has a tendency to puff up so that it is necessary to decrease the vacuum. When the desired reduction in moisture has been reached, the vacuum is suddenly increased. This action causes the mass to expand explosively and to fill the entire kettle. Dried in this manner, malted milk has a brittle honeycombed structure and a moisture content of about 3 per cent. It is this final evaporation in the kettle, which requires about 4 hours, that gives malted milk its characteristics.

CONCLUSIONS.

From the data presented in this paper, it is concluded that genuine malted milk has well-defined, readily recognizable characteristics that

differentiate it from other products of similar composition. These characteristics, it is believed, can be obtained only through evaporation in vacuo of the component parts, malt infusion, and whole milk. The experiments point to a slight proteolytic digestion during the manufacturing process; lipolytic digestion apparently does not occur. Malted milk is diastatically active. Its protein digestive qualities are not pronounced, although experiments indicate that reactions of this character may take place to a slight extent.

THE MICROSCOPICAL IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS.

By F. HILLIG and B. G. HARTMANN (Food Control Laboratory¹, Food, Drug and Insecticide Administration, U. S. Department of Agriculture, Washington, D. C.).

In the preceding paper, "Malted Milk—A Study of the Action of the Enzymes of Malt upon Milk Solids during Manufacture", the physical and biological properties of malted milk are discussed. It is stated therein that the product resulting from the evaporation in vacuo of a specially prepared "malt-flour infusion" and whole milk has characteristics that are readily discerned. It is pointed out, furthermore, that the evaporation in vacuo is necessary to obtain the product that is recognized in the trade as malted milk.

Recently a product has been marketed under the label "malted milk". Although it contains the required quantity of butterfat and apparently is produced from a malt infusion-milk mixture by drying, it does not show the general characteristics of genuine malted milk; physically and organoleptically it does not even resemble malted milk. It is light yellow in color and has a fluffy appearance and a distinctly tallowy odor; the characteristic aroma and taste of malted milk are entirely lacking.

Obviously it is possible for two materials to have a similar chemical composition and still be entirely different in their physical properties. Therefore, to judge the genuineness of a malted milk, the writers considered it necessary to find some other means than that of chemical analysis to differentiate between genuine malted milk and products of similar chemical composition.

In 1918 Ballard² discussed the microscopy of malted milk and its components, and it was his work that suggested this method for purposes of identification.

It would be expected that the components of malted milk, malt extract and milk solids, would be united into homogeneous masses because the

¹ R. W. Balcom, chemist in charge.

² J. Am. Pharm. Assoc., 1918, 7: 326.

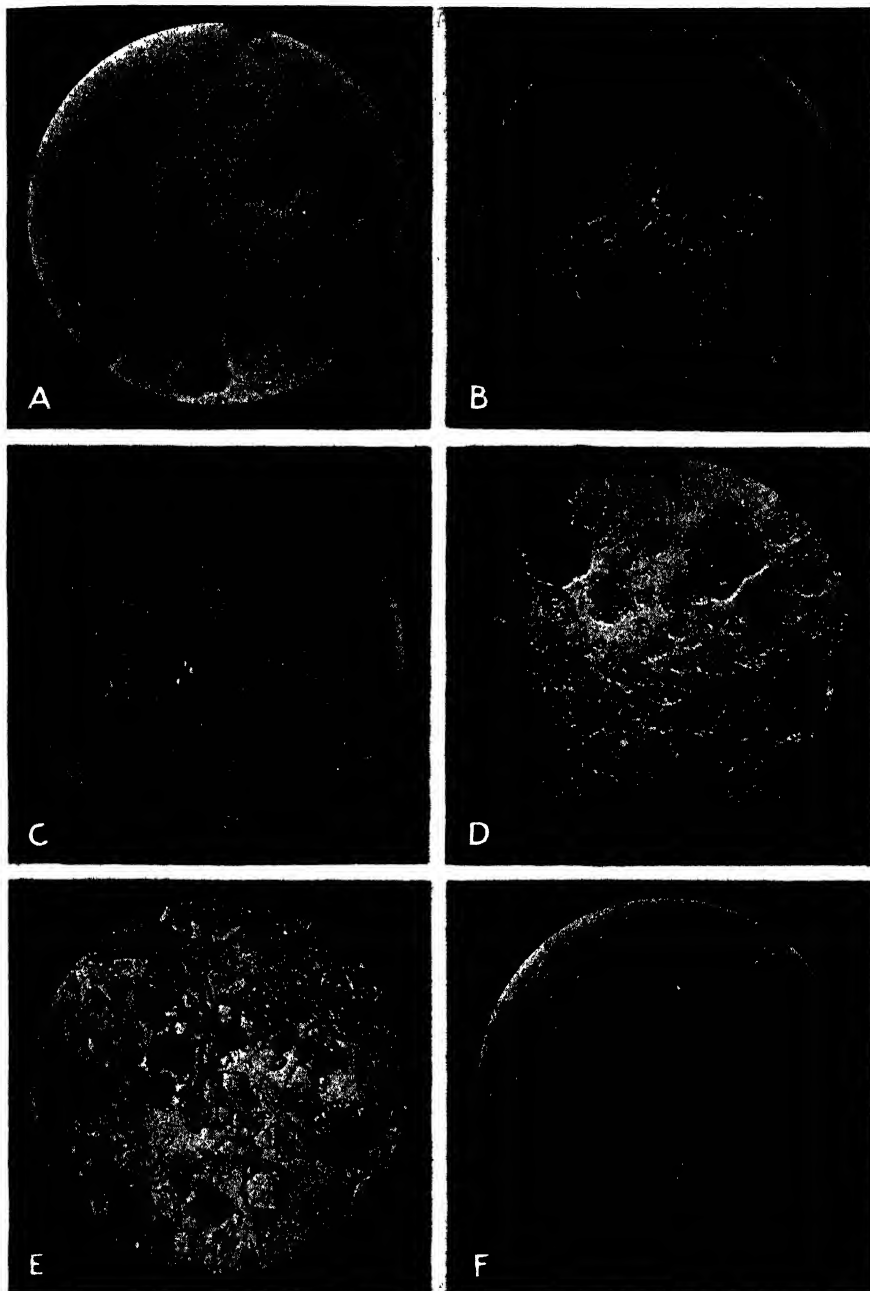


PLATE 1.

FIGURE A.—SPRAY-DRIED WHOLE MILK; (a) MILK MASSES, (b) FAT GLOBULES.

FIGURE B.—SPRAY-DRIED MALT EXTRACT.

FIGURE C.—MECHANICAL MIXTURE; (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES.

FIGURE D.—SPRAY-DRIED SKIM MILK.

FIGURE E.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR.

FIGURE F.—DRUM-DRIED MALT EXTRACT.

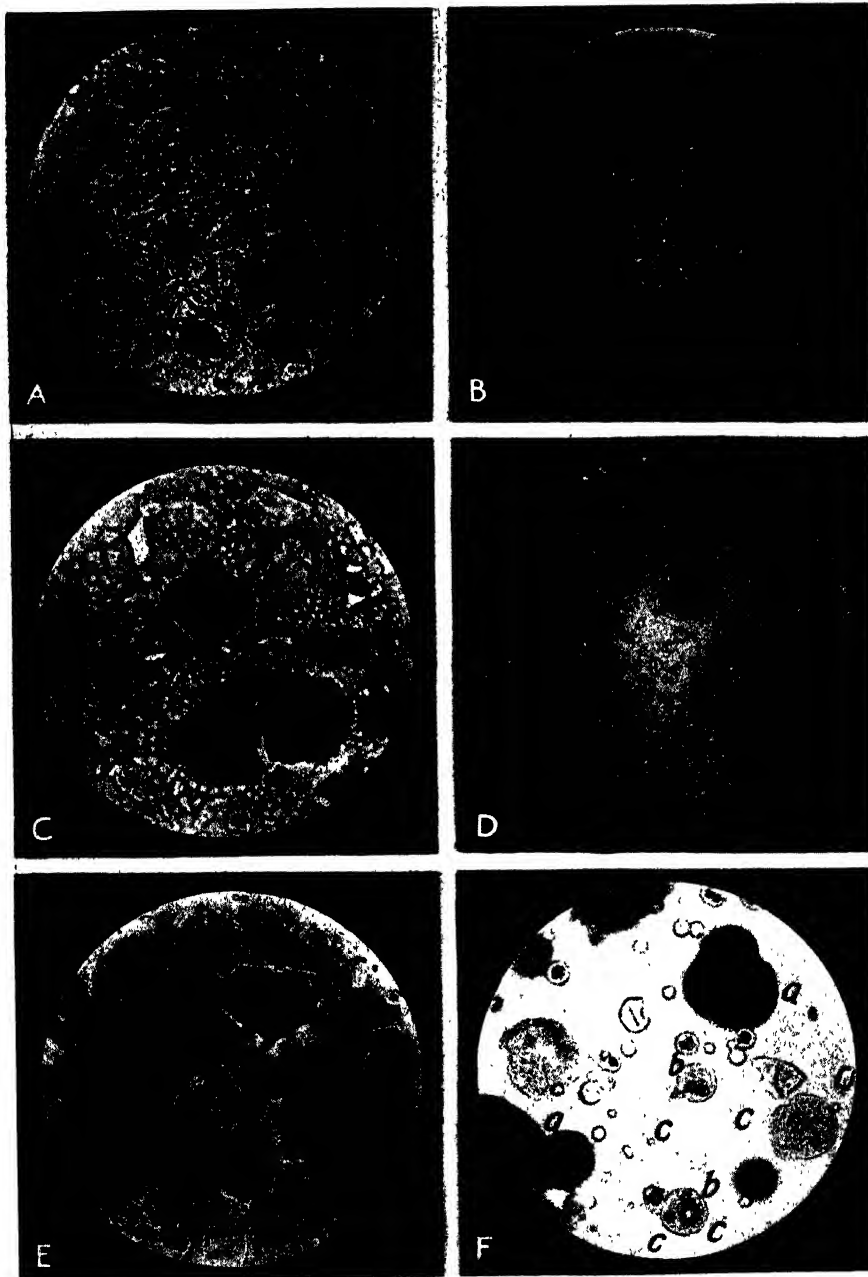


PLATE 2.

FIGURE A.—MECHANICAL MIXTURE; (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR.

FIGURE B.—MALTED MILK

FIGURE C.—SWEET CHOCOLATE FLAVOR MALTED MILK; (a) MALTED MILK MASSES, (b) SUGAR.

FIGURE D.—MECHANICAL MIXTURE; (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR.

FIGURE E.—MECHANICAL MIXTURE; (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR.

FIGURE F.—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING; (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

material is kept in constant agitation during the final evaporation to dryness. On the other hand, it would be expected that as a result of simple mechanical mixing the component parts would still retain their individual identities. The study here recorded is based upon these considerations.

In order to make the study complete it was necessary to establish the microscopical identity of genuine malted milk, of mechanical mixtures of the ingredients, as well as the characteristics of the flavored articles containing these two products as basic ingredients. For the preparation of the photomicrographs genuine malted milk, spray-dried whole and skim milk, spray-dried and drum-dried malt extract, cocoa and sugar and mixtures of certain of these ingredients were used. The majority of the exhibits were prepared from samples obtained in the market. The slides were prepared by mounting the materials in heavy mineral oil. A magnification of 170-200 diameters was used.

DESCRIPTION OF THE PHOTOMICROGRAPHS.

PLATE I.

Figure A represents a spray-dried whole milk. The large particles represent aggregates of globular milk masses having a stippled surface (a). The fat appears as droplets (b).

Figure B represents a spray-dried malt extract having the appearance of aggregates of droplets enclosed in spherical masses.

Figure C represents a product made by mixing the spray-dried whole milk and the spray-dried malt extract, shown in Figures A and B in the proportion necessary to give the approximate composition of malted milk. The globular stippled milk masses (b) and the malt extract masses (a) are easily recognized.

Figure D represents a spray-dried skim milk, which might be confused with the spray-dried malt extract (Figure B) because the structure of the spherical masses is similar. A comparison of the two pictures, however, will show that the droplets in the malt extract masses are larger than those appearing in the milk masses.

Figure E represents a product purchased on the market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt extract (b), dried skim milk (a), cocoa (c) and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under the microscope. The highly refractive, irregular fragments of sugar cannot be mistaken.

Figure F represents a drum-dried malt extract. It consists of clear, highly refractive fragments closely resembling broken glass.

PLATE II.

Figure A represents a mechanical mixture of dried whole milk (Figure A, Plate 1), dried malt extract (Figure B, Plate 1), cocoa and sugar.

The milk (a) and malt extract (b) masses and the sugar particles (d) are readily recognizable. A mass of cocoa appears near the center of the picture (c).

Figure B is characteristic of the genuine malted milks of the market. This picture cannot be mistaken for any product of similar composition. The malt extract solids and the milk solids are incorporated into homogeneous irregular fragments having a stippled surface.

Figure C represents a "sweet-chocolate-flavor malted milk" purchased on the market, prepared by simultaneously evaporating in vacuo milk, malt infusion, cocoa and sugar. It is easy to recognize the characteristic malted milk masses (a), shown in the picture immediately preceding. They are slightly thicker and appear darker in the picture because the cocoa is intimately associated with them.

Figure D represents a mechanical mixture of spray-dried skim milk (Figure D, Plate 1), spray-dried malt extract (Figure B, Plate 1), cocoa and sugar. No trouble is experienced in identifying these materials in the picture.

Figure E represents a mechanical mixture of malted milk (Figure B, Plate 2) cocoa and sugar. Examination shows that malted milk (a) is present.

Figure F represents the product described in the text as found on the market under the label "malted milk". It shows none of the characteristics found in genuine malted milk, as is readily seen by a comparison with Figure B, Plate 2. Individual milk masses (a), fat globules (c), and malt extract masses (b) closely resembling the spray dried products (Figures A and B, respectively, Plate 1) predominate. Some particles show the stippled surface of genuine malted milk, but they are spherical instead of angular. A comparison of this picture with the picture showing the mechanical mixture of spray-dried whole milk and spray-dried malt extract (Figure C, Plate 1) will show a striking similarity.

The authors wish to express their thanks to G. L. Keenan of the Microchemical Laboratory, Food, Drug and Insecticide Administration, Department of Agriculture, for the preparation of the photomicrographs and also for helpful suggestions in preparing the material.

THE CHLORATE METHOD FOR THE DETERMINATION OF NITRATE NITROGEN, TOTAL NITROGEN, AND OTHER ELEMENTS IN SOILS AND PLANT TISSUES¹.

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The nitrogen content of various tissues and of soils is usually determined by converting the nitrogen to ammonia and distilling from sodium hydroxide. If the nitrate nitrogen is to be determined, a separate, and in

¹ Published by permission of the Director of the Kentucky Agricultural Experiment Station.

the case of plant tissue a laborious, determination is necessary. No general methods are in use by which the nitrogen is oxidized to its highest valency and then distilled off as nitric acid.

It has been suggested by the writer¹ that chloric acid (HClO_3) or its sodium salt would be an excellent oxidizing agent, provided its explosive tendencies could be controlled. As a result of preliminary tests on different samples it was found that with it nitrate and total nitrogen determinations could be made in much less time than by the regular Kjeldahl method and that the results compared very favorably. At the same time a solution of the inorganic elements contaminated only by sodium sulfate and sulfuric acid was obtained. As a result of more extended investigation and refinement of method the writer finds it possible to determine nitrate and total nitrogen accurately and quickly and to leave the residue in such a state that the other inorganic elements also may be determined easily.

The methods in the various determinations, the reactions involved, and some of the results secured follow.

DETERMINATION OF NITRATE NITROGEN BY DISTILLATION.

Grind the dry samples to pass a 50-mesh sieve, but leave the green tissue intact. Take care to preserve the nitrate as such, because enzyme and microbic action may carry on reduction processes after the sample has been removed from its natural environment. Place immediately in a 500 cc. Kjeldahl flask a sufficient quantity of the sample to yield 0.5–1.0 mg. of nitrate nitrogen (usually 1.0–3 grams of soil or green tissue and 0.2–0.5 gram of dry tissue). Wash down the sides of the flask with 25 cc. of 50 per cent by volume sulfuric acid. Connect the flask to an ordinary glass water-cooled condenser, the free end of which leads through a two-holed rubber stopper into a 200 cc. cylindrical absorption tower containing 150 cc. of freshly prepared chlorine dioxide solution. Prepare this solution by dropping concentrated hydrochloric acid on 15 grams of sodium chlorate in a 250 cc. Erlenmeyer flask fitted with a dropping funnel and delivery tube. Pass the yellow gas evolved into water until a deep yellowish-green color develops. Prepare a fresh solution for each determination because chlorous and chloric acids form on standing and decompose the phenoldisulfonic acid used later.

To expose the gases as long as possible to the chlorine dioxide, fit the exit hole from the tower with an ordinary Kjeldahl trap (Hopkins form) adjusted to such a depth that a portion of the tower contents will be forced into it.

Heat the sample in the Kjeldahl flask sufficiently to expel the gases rapidly but not violently into the absorption tower and then out through the liquid in the trap. Rotate the flask gently to cover all particles of sample with hot acid. Allow distillation to proceed rapidly as soon as the water vapor begins to condense, foaming ceases, and little gas is evolved, and continue until white fumes begin to be evolved. Remove the connection to the Kjeldahl before turning out the flame. Save the residue for the determination of reduced nitrogen if this determination is to be made.

Wash out the condenser several times with a few cc. of water. Wash out the trap and tubes. If the resulting solution in the absorption tower, which should be yellow, gets colorless at any time, add fresh chlorine dioxide solution until it remains yellow. Immediately transfer the solution to a 500 cc. Erlenmeyer flask and boil until colorless. Build up the solution to a known volume, and to an aliquot containing at least 0.25 mg.

of nitrate nitrogen add while hot 0.05–0.1 gram of silver sulfate and shake well. Allow to stand 5 minutes with occasional shaking. Then add 0.5–1 gram of calcium oxide and shake. After allowing to stand a few minutes with intermittent shaking, filter, pouring the mixture back on the filter until a clear filtrate is obtained. Evaporate to complete dryness without spattering or burning an aliquot large enough to contain at least 0.2 mg. of nitrate nitrogen. Add 2–3 cc. of phenoldisulfonic acid and cover all salts. Let stand 5–10 minutes. Wash down the sides with 20–30 cc. of water and shake until most of the salts dissolve. Gradually add 25 per cent sodium hydroxide until a yellow color develops. Add a few cubic centimeters in excess.

Make up to 100 cc. and shake with 0.5 gram of calcium hydroxide. Filter off enough solution to read against a standard containing 0.0025 mg. of nitrogen per cubic centimeter. Adjust the volume of the unknown until its strength is nearly equal to that of the standard. Calculate the nitrate nitrogen present in the sample from the colorimeter readings.

TABLE 1.
Nitrate nitrogen by the distillation method.

SAMPLE	DETERMINATION NO. 1	DETERMINATION NO. 2	OFFICIAL METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Tomato Leaves* (dry No. 8)	0.40	0.44	
Artificial Manure*	0.08	0.072	
Fresh Green Corn Stalks*	0.019	0.020	
Soil No. 1*	0.08	0.072	0.075
Soil No. 2	0.01	0.01	0.065
Soil No. 3	0.0027	0.0024	0.008

* Determinations made in the Soils Laboratories of the Iowa Experiment Station.

TABLE 2.
Recovery of nitrate nitrogen added.

SAMPLE	NITRATE NITROGEN IN ORIGINAL SAMPLE	NITRATE NITROGEN ADDED	TOTAL FOUND
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
0.2 gram tomato leaves (No. 8)* + 0.5 mg. nitrogen as NO_3	0.82	0.5	1.28
0.5 mg. nitrogen in ClO_2 treated as distillate*		0.5	0.52
1 mg. nitrogen in 50 per cent H_2SO_4 and distilled into ClO_2		1.00	1.06 1.08

* Determinations made in the Soils Laboratories of the Iowa Experiment Station.

The results in Table 1 are also checked by determinations on reduced and total nitrogen (see Table 4) since nitrate nitrogen plus reduced nitrogen should equal total nitrogen. The results show that in some

cases nitrate nitrogen is undoubtedly completely caught, while in others some is lost, depending probably on the nature of the substances in the sample that reduce the nitric acid before it leaves the sulfuric acid solution, and also on the rate at which the gases are expelled. Double absorption towers might remedy the loss. It was noted in soil samples Nos. 2 and 3, where much nitrate nitrogen was lost, that gaseous evolution was exceptionally violent. These samples were from clay loam soils as contrasted with the black humus soil of sample No. 1, in which complete recovery of nitrate nitrogen was secured.

Reactions typical of those involved in the determination are as follows:

- (1) $\text{KNO}_3 + \text{H}_2\text{SO}_4 \rightarrow \text{HNO}_3 + \text{KHSO}_4$.
- (2) 2HNO_3 (in presence of reducing material) $\rightarrow \text{H}_2\text{O} + 2\text{NO} + 3\text{O}$.
- (3) $8\text{NaClO}_3 + 24\text{HCl} \rightarrow 8\text{NaCl} + 12\text{H}_2\text{O} + 9\text{Cl}_2 + 6\text{ClO}_2$.
- (4) NO on entering the ClO_2 solution is likely reacted on as follows:
 $4\text{NO} + 2\text{ClO}_2 \rightarrow 4\text{NO}_2 + \text{Cl}_2$,
 $2\text{NO}_2 + \text{H}_2\text{O} \rightarrow \text{HNO}_3 + \text{HNO}_2$,
 $4\text{HNO}_2 + 2\text{ClO}_2 \rightarrow 4\text{HNO}_3 + \text{Cl}_2$,
 $2\text{NO} + \text{Cl}_2 \rightarrow 2\text{NOCl}$,
 $\text{NOCl} + \text{H}_2\text{O} \rightarrow \text{HCl} + \text{HNO}_2$.

On boiling, excess chlorine and chlorine peroxide, but not chloric and chlorous acid formed by water and chlorine peroxide are driven off, and a clear dilute solution of hydrochloric and nitric acid with small quantities of sulfuric acid formed by oxidation of sulfurous acid are left.

In fresh solutions of chlorine peroxide very little hydrolysis takes place, but on long standing considerable chloric and chlorous acids form. These would decompose the phenoldisulfonic used later; therefore, fresh solution must be used.

On adding silver sulfate the chloride is thrown down almost completely as silver chloride. Addition of calcium oxide or calcium hydroxide converts excess silver to insoluble silver oxide, and all nitric acid is combined as calcium nitrate, which is stable up to 500°C . The silver chloride, silver oxide, and excess calcium hydroxide are filtered off, and a clear solution of calcium nitrate, calcium hydroxide, and calcium sulfate, which is ideal for the determination of nitrate nitrogen, is left. Emerson¹ has listed the reactions involved in the phenoldisulfonic acid determination of nitrate.

DETERMINATION OF REDUCED NITROGEN.

If the residue saved from the distillation of nitrate nitrogen is to be used, cool it to nearly room temperature and add 10 cc. of water. Cool again to about room temperature. If a fresh sample is to be used, take a smaller quantity than that used for the determination of nitrate nitrogen, regulating the size according to the amount of nitrogen in the sample. Add 20 cc. of 50 per cent (by volume) sulfuric acid and boil until the sample is well carbonized and it is certain nitrate nitrogen is driven off (approximately 5 minutes). Cool, add 10 cc. of water, and cool again to about room temperature.

¹ Soil Characteristics, p. 134.

Add 1 gram of sodium chlorate for each 0.1 gram of dry tissue, or for each 0.5 gram of green tissue or soil. Shake well and place in exactly the same apparatus as that described under nitrate nitrogen. Before connecting, heat the sample rapidly until the green fumes of chlorine peroxide first evolved are decomposed with a slight explosion and only white fumes are present. If excessive green fumes appear, take special precautions because a violent explosion will take place when the temperature approaches 100°C. (In all ordinary samples only small quantities of greenish yellow chlorine peroxide are formed if the flask is heated rapidly. This is especially noticeable if the flame is adjusted so that the unburned gases in the center of the flame play against the bottom of the flask while the sides of the flask above the solution are hot and decompose the chlorine peroxide before much is formed. Above 100°C. no more will form, but violent action continues, and the flame should be lowered as soon as it is certain 100°C. has been reached.) Connect immediately to the distillation apparatus. After violent reaction ceases, distil rapidly into water contained in the absorption towers. (The water may be made slightly yellow with chlorine peroxide prepared as for nitrate nitrogen, but in most cases water alone seems to absorb all the nitric acid coming over.) Disconnect the Kjeldahl flask as soon as the solution remains colorless, white fumes form, and sulfuric acid condenses on the sides, since all water and nitric acid are then distilled over. Take special care to guard against sucking back because any cooling of the flask before disconnecting will cause an explosion if water is sucked back into the hot acid. Always disconnect *before* turning out the flame. (The entire distillation usually takes from 15 to 30 minutes.) From this point proceed exactly as directed for the determination of nitrate nitrogen, except to use aliquots to secure about 0.2 mg. of nitrogen. If the solution does not clear, indicating that not enough chlorate was used, distil off all the water and cool. Add more water and sodium chlorate and redistil the solution. (No nitrogen is lost because the sulfuric acid holds all unoxidized nitrogen as ammonium sulfate until more chlorate is added.)

TABLE 3.
Reduced nitrogen.*

SAMPLE	DETERMINATION NO. 1	DETERMINATION NO. 2
	<i>per cent</i>	<i>per cent</i>
Tomato Leaves No. 8	3.32	3.25
Artificial Manure	3.32	3.25
Fresh Green Corn Stalks	0.030	0.033
Soil No. 1	0.32	0.35

* Data secured in Soils Laboratories of the Iowa Experiment Station.

In another experiment, 4.5 mg. of ammonia nitrogen in the form of ammonium nitrate was added to Sample No. 8, and 3.9 mg. of reduced nitrogen was recovered. Some nitrogen may have been lost as nitrogen monoxide.

REACTIONS INVOLVED IN THE DETERMINATION OF REDUCED NITROGEN.

When nitrogenous organic matter is heated with 50 per cent (by volume) sulfuric acid the reduced nitrogen is caught as ammonium sul-

fate, while nitrate and nitrate nitrogen distil off. In the reactions involved with sodium chlorate a part of the nitrogen probably is oxidized directly to nitric acid, while some is caught as ammonium sulfate and then oxidized to nitric acid. If in the distillation any of this acid decomposes, it is converted back by the chlorine peroxide solution. However, it seems that in the presence of chloric acid all the nitric acid distills over without decomposition. It is not necessary to connect the Kjeldahl flask to the distillation apparatus until the chlorine peroxide decomposes, because this process takes place at about 100°C. and nitric acid does not come off until considerably higher temperatures are reached. This is a great advantage since the explosion of chlorine peroxide in the closed flask would be dangerous. However, relatively large quantities can explode in an open Kjeldahl flask without harm. After 100°C. is reached the chlorine peroxide breaks down as fast as it is formed.

DETERMINATION OF TOTAL NITROGEN.

Put into a dry 500 cc. Kjeldahl flask a smaller sample than that used in determining nitrate nitrogen, regulating the quantity according to the nitrogen content. Add sodium chlorate at the same rate as for reduced nitrogen and wash down the sides with 25 cc. of 50 per cent by volume sulfuric acid. Heat and distil as in the determination of reduced nitrogen, taking care to prevent great masses of chlorate and sample from collecting directly over the flame and causing violence. (Gentle rotation will prevent this and will also wash down particles from the sides of the flask, thus bringing all the sample into the reaction, to assure complete oxidation.) Determine nitrate nitrogen in the distillate as for the nitrate nitrogen determination and calculate the quantity of total nitrogen.

TABLE 4.

Comparison of results on nitrate plus reduced nitrogen and total nitrogen.

SAMPLE	DETERMINATION	TOTAL NITROGEN	NITRATE NITROGEN	REDUCED NITROGEN	TOTAL NITROGEN—SEPARATE DETERMINATION	TOTAL NITROGEN—KJELDAHL METHOD
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Sample No. 8, Tomato Leaves*	1	2.72	0.42	2.20	2.62	
	2	2.80				
	3	2.63				
	4	2.75				
	5	2.75				
	6	2.80				
Artificial Manure*		3.4	0.078	3.29	3.37	3.46
Soil No. 1*	1	0.40	0.076	0.335	0.411	
	2	0.44				
	3	0.40				
	4	0.42				

* Data secured in Soils Laboratories of the Iowa Experiment Station.

TABLE 5.

Comparison of results on total nitrogen obtained by the new method and the Kjeldahl method.

SAMPLE	DETERMINATION	NEW METHOD	KJELDAHL METHOD
Sweet Clover Hay*		<i>per cent</i> 2.10	<i>per cent</i> 2.20
Manure*		1.80	1.92
Tomato Leaves: No. 1	1 2	3.88 4.00	3.91 3.96
No. 2	1 2	3.25 3.50	3.39
No. 3	1 2	3.75 3.88	3.82
No. 4	1 2	3.50 3.50	3.47
No. 5	1 2	3.58 3.88	3.36
No. 6	1 2	3.50 3.50	3.48
Tomato Stems: No. 1	1 2	2.82 2.82	2.89 2.86
No. 2	1 2	2.75 2.68	2.75 2.72
No. 3	1 2	2.38 2.25	2.65 2.42

* Data secured in Soils Laboratories of the Iowa Experiment Station.

To test the recovery of nitrogen in ammonium nitrate a solution of this salt containing 9 mg. of nitrogen was added to 0.2 gram of Sample 8, green tomato leaves, being an addition of 4.5 mg. of ammonia nitrogen and 4.5 mg. of nitrate nitrogen. The determination gave 14.2 mg. of total nitrogen. The sample itself contained 5.5 mg. of nitrogen, and 9 mg. was added to make a total of 14.5 mg.

PREPARATION OF THE CLEAR RESIDUE FOR THE DETERMINATION OF NON-VOLATILE ELEMENTS.

Save the clear residue in the Kjeldahl flask for the determination of any non-volatile element or elements except sodium and sulfur. Tilt the flask slightly downward, but not so much that the contents run out. Support the end of the neck on a beaker held in place by some device. Then distil the residue rapidly. (The sulfuric acid condenses in the neck and runs into the beaker.) Continue the distillation until all the sulfuric acid is driven off and only a melt of sodium sulfate, sodium acid sulfate, and the minerals or ash of the plant is left. Allow to cool. Dissolve the residue in hot water and make to a known volume for aliquotting or add a reagent directly, depending on the deter-

minations desired. If a determination of sodium is desired, use potassium chlorate in place of sodium chlorate.

POSSIBILITY OF DETERMINING TOTAL CARBON.

While determining total nitrogen with sodium chlorate it was noticed that a large quantity of gas was evolved and that carbon entirely disappeared. It is obvious that a good share of this gas is carbon dioxide. It comes off so rapidly, however, that potassium hydroxide bulbs will not absorb it all. The following procedure was tried.

A 2 liter Florence flask was filled with acidulated water and connected to the absorption tower used for collecting nitrogen. Another tube led from this flask to a 3 liter flask. When the gases were evolved through the absorption tower they displaced the water from the 2 liter flask to the 3 liter flask, and all the gases were caught. The system was disconnected from the condenser after complete distillation and connected to the following absorption train:

- (1) An absorption tower containing a saturated solution of potassium iodide, acidified with sulfuric acid to catch chlorine.
- (2) A small bubble tube containing silver nitrate in nitric acid to note whether traces of chlorine were coming over.
- (3) A U-tube containing phosphorus pentoxide and glass wool to absorb water vapor.
- (4) A weighed Fleming soda-lime and phosphorus pentoxide bulb as used in the regular total carbon apparatus.
- (5) A sulfuric acid scrubber.
- (6) A bottle of barium hydroxide to indicate loss of carbon dioxide.

A graduated cylinder filled with water was attached to the 3 liter flask in such a way that when gas was drawn out of the system water was drawn out of the graduated cylinder, and the quantity of gas drawn out could be determined. The absorption train as outlined above was attached to the flasks and cylinder, and the entire system was attached to a suction pump. The Fleming bulb was taken out, and the train was filled up to the bulb with gas from the flask by suction. The Fleming bulb and the other part of the train were then inserted, and several hundred cubic centimeters of gaseous mixture from the flask was drawn through the train at about 40-50 cc. per minute. The gain in weight of the Fleming bulb was noted, and the volume of water forced into the 3 liter flask during the distillation was measured and taken as the total volume of gas forced over.

From these data the quantity of total carbon in the sample was calculated. Several aliquots of gas from the same sample could thus be used. The results secured checked to within 0.2 per cent, which was considered good for the large quantity of carbon present. However, time did not permit further analyses, and this method is only submitted as having possibilities. This sort of setup might make possible the complete absorption of nitric oxide, which escapes reaction in chlorine peroxide in the determination of nitrate nitrogen by distillation.

THE DETERMINATION OF THE SAPONIFICATION NUMBER—A MORE STABLE ALCOHOLIC POTASH REAGENT.

By D. T. ENGLIS and V. C. MILLS (University of Illinois, Urbana, Ill.),

INTRODUCTION.

One of the difficulties encountered in the determination of the saponification number of fats and oils is the maintenance of a somewhat permanent colorless reagent. The usual solution of potassium hydroxide in 95 per cent ethyl alcohol, owing to the formation and resinification of the aldehyde, rapidly turns yellowish and finally brownish red, so that the indicator change in the titration of the excess alkali is detected with great difficulty. The purification of the alcohol by treatment with silver nitrate and alkali, according to the method of Dunlap¹, makes possible the preparation of a solution of greater stability, but eventually oxidation and discoloration take place. Gill² states that scarcely any darkening occurs if the solution is kept under an atmosphere of hydrogen. However, there are certain inconveniences to this method.

The phenomenon of oxidation relative to the catalytic effect of hundreds of compounds has been studied by Moureu and Dufraisse³, and a review of their experimental work and their theories of anti-oxygenic activity has recently appeared. A common property of all anti-oxygens that appears essential is oxidizability. Some considerations in this connection gave rise to the idea that sodium hydrosulfite might be effective in maintaining a colorless solution of alcoholic alkali. It seemed probable that the reducing nature of the material would inhibit the oxidation of the alcohol or perhaps, if some aldehyde was produced, the sulfite present would form an addition product with it.

PREPARATION AND PRELIMINARY TESTING OF THE REAGENT.

To 2 liters of alcoholic potash reagent, prepared according to the official method, 10 grams of sodium hydrosulfite was added; the solution was then shaken and allowed to stand. Except for an occasional shaking the solution remained undisturbed in a stoppered 2 liter flask for about a year. At the end of this time, it being practically colorless and giving promise of success, a number of preliminary determinations of the saponification value of several oils were run. Apparently normal values were obtained. It was then decided to make a comparison with the official reagent.

It should be emphasized in the beginning that the hydrosulfite must be allowed to settle out completely and the clear supernatant liquid be pipetted off for use. The material settles readily and is sufficiently insoluble in the alcohol to have no effect upon the determination. The

¹ *J. Am. Chem. Soc.*, 1906, 28: 397.

² *Oil Analysis*, 10th ed., 1922, p. 71.

³ *Chemical Reviews*, 1926-7, 3: 113.

addition of 0.2 gram of the salt to the saponification flask lowered the apparent saponification value about 20 units. If the alcoholic potash solution has already become colored, addition of the hydrosulfite will not bring about decolorization except on very long standing (several months at room temperature).

COMPARISON OF MODIFIED AND OFFICIAL REAGENT.

The official reagent was prepared in the regular way¹, as was the modified reagent, except that about 5 grams per liter of sodium hydrosulfite was added immediately after making up. The solution was shaken and the solid material was allowed to settle. Portions of the clear supernatant liquid were then withdrawn by a pipet. The determinations were carried out according to the official procedure. Water-cooled Hopkins condensers were substituted for air condensers to reduce the loss of solvent. The oils used and results obtained are indicated below. The values given are an average of three or more determinations. The results of the experiments show no significant differences between the reagents.

	OFFICIAL REAGENT	MODIFIED REAGENT
Raw Linseed Oil.....	192.9	192.4
Cottonseed Oil	195.1	195.3
Corn Oil.....	189.8	189.9

DISCUSSION.

Part of the difficulty in securing good agreement for saponification numbers may be due to incomplete reactions.

The experiments carried out by Pardee, Hasche and Reid² show the varied rates of hydrolysis of some of the common oils. In the experiments made by the writers the linseed oil sample was far from completely saponified at the end of the suggested approximate time (30 minutes). The reagent, however, was a little less than half normal.

Certain desirable features in the use of butyl alcohol and iso-propyl alcohol instead of ethyl alcohol as a solvent for the potassium hydroxide have been pointed out by Pardee, Hasche and Reid and by Schuette and Harris³.

The fact that these solvents are less subject to oxidation and discoloration is especially pertinent here. The writers have used butyl alcohol with good success on some occasions. However, when the back titration was large and a separation into alcohol and aqueous layers took place, the approach to the end point had to be made more slowly, and greater difficulty was experienced with the titration than with the ethyl alcohol reagent.

¹ *Methods of Analysis*, A. O. A. C., 1925, p. 288.

² *J. Ind. Eng. Chem.*, 1920, 12: 481.

³ *J. Am. Pharm. Assoc.*, 1926, 15: 166.

INHIBITION OF COLOR DEVELOPMENT DURING THE SAPONIFICATION PROCESS.

Since the hydrosulfite was effective in preventing the color development in the reagent, an effort was made to extend this type of action to the saponification procedure itself. A number of experiments were carried out with various oils in which small quantities of hydroquinone, pyrogallol, resorcinol, picric acid and tannic acid were added to the saponification flask. No general beneficial effect was observed. The production of color may be due to a great many types of action of which oxidation is but one, and a general inhibitor is not probable.

SUMMARY.

It has been found that the addition of a small quantity of sodium hydrosulfite (5 grams) to an alcoholic potash reagent is effective in preventing coloration of the solution. When the clear supernatant liquid is used for the determination of saponification values the results obtained with this reagent agree with those obtained with the official reagent.

An attempt to find a general inhibitor for color development during the saponification process was not successful.

NOTE.

Shortening the Time of Nitrogen Determinations by the Use of the Grid Burner.¹—In a recent article Shedd² showed that copper sulfate was not so effective a catalyst as mercury for a short digestion (20 minutes), but that for the ordinary period (3–5 hours) it was just as satisfactory for materials such as feeding stuffs. He showed that with mercury as a catalyst the 20-minute digestion over a grid burner was satisfactory for all kinds of feeding stuffs tried, but that when the same samples were tried and copper sulfate used instead of mercury, the results were not reliable.

In the usual routine followed in this laboratory, Bunsen burners are used, with natural gas as a source of heat, and the samples are digested from 3 to 5 hours, depending upon their character. As copper sulfate instead of mercury is preferred as a catalyst, it occurred to the writers that possibly the digestion period could be shortened by using a grid burner³. This was done, and the results given in the table, which were obtained on general laboratory samples, are so conclusive that it is believed a digestion of 1½ hours is satisfactory. A longer digestion with the grid burner involves loss of too much acid; therefore it is essential to terminate the digestion at the end of the 1½ hour period. This is not necessary or even desirable in the digestion over the Bunsen burner.

As a result of this investigation, the grid burner is used for the digestion of the samples that must be reported at the earliest possible time.

¹ Contribution from the Department of Feed Control, Kentucky Agricultural Experiment Station, September 24, 1928, by H. D. Spears and W. G. Terrell.

² *This Journal*, 1927, 10: 507.

³ A Fisher burner was used.

Comparison of the grid burner with the Bunsen burner for Kjeldahl digestions.

MATERIAL	PERCENTAGE OF PROTEIN							
	Bunsen Burner		Grid Burner					
	3 hours	4 hours	¼ hour	½ hour	¾ hour	1 hour	1½ hours	1½ hours
Dairy Feed	23.20		22.10					
Shorts	12.00		11.45					
Hog Feed	17.75		17.10					
Egg Mash	21.10			20.60				
Egg Mash	17.00			17.15				
Hog Ration	15.55			15.50				
Mixed Feed	10.30			10.10				
Tankage		48.10			47.60			
Dairy Feed	21.40					21.40		
Dairy Feed	20.15					19.50		
Dairy Feed	17.50					17.30		
Dairy Feed	16.80					16.75		
Mixed Corn	8.70					8.60		
Mixed Wheat								
Feed	13.30					13.40		
Mixed Wheat								
Feed	14.00					13.95		
Winter Wheat								
Mixed Feed	11.80					11.80		
Laying Mash	19.80					19.80		
Sheep Feed	13.40					13.20		
Cottonseed Meal		38.20				38.20		
Cottonseed Meal		40.35				40.55		
Mixed Wheat								
Feed	14.70						14.50	
Shorts	15.10						14.90	
Cottonseed Meal	39.50						39.10	
Wheat Germ	28.70							28.70
Growing Mash	13.10							13.00
Corn Feed Meal	10.10							10.00
Raw Bone Meal	24.00							23.60
Dairy Feed	23.50							23.50
Horse Feed	8.60							8.70
Cottonseed Meal		38.25						38.05
Cottonseed Meal		39.35						39.30
Cottonseed Meal		40.40						40.80
Cottonseed Meal		37.25						37.30
Mixed Wheat								
Feed	15.35							15.40
Horse and Mule								
Feed	7.30							7.25
Shipstuff	13.10							13.20
Growing Mash	15.20							15.60
Alfalfa Meal, Oat								
Hulls	8.55							8.57
Corn Chop	8.85							8.69
Dairy Feed	16.50							16.86
Laying Mash	20.20							20.40
Horse Feed	9.90							9.95
Bat Guano	9.47*							9.50*
Tankage, I		53.05						52.65
II		52.50						52.55
III		52.80						53.15

* Percentage of nitrogen

NEW BOOKS.

Soil Management. By Firman E. Bear. John Wiley and Sons, Inc., New York. Price \$3.50 net. Second Edition. An excellent text book containing 380 pages treating: requirement of crops, characteristics of soils, utilizing soil resources, and supplementing soil resources. "The purpose of this book is primarily that of acquainting the student with the applications of those scientific facts and principles that are of use in planning constructive systems of soil management and increasing the productive capacity of soils". However, it is a beginner's course and not for advanced students.

Text Book of Inorganic Chemistry. Vol. X. The Metal Amines. By M. M. J. Sutherland. J. B. Lippincott Co., Philadelphia, Pa., 1928. Price \$10.00. This is next to the last of a series edited by J. Newton Friend, "A compilation of the main points relating to this large class of substances". The order is that of the periodic system, although the first three chapters are general; the next six chapters cover, separately, groups I to VII; the amines of iron, cobalt and nickel; ruthenium, rhodium and palladium, osmium, iridium and platinum are treated in three concluding chapters. Author and subject indexes are very complete.

Fruit Pectin. By William A. Rooker, Avi Publishing Co., Inc., New York, 1928. Price \$6.00. This book of fifteen chapters explains the methods of removing that quantity of pectin from fruits that is "economically justifiable", describes manufacturing operations, and gives the various commercial uses of this product. Tested formulas, interesting data and patents covering pectin manufacture are given. More general references to the work of other specialists and workers in this field of chemistry would make this book of more value to the chemist interested in this product.

Critical Studies in the Legal Chemistry of Foods. By R. O. Brooks. The Chemical Catalog Company, Inc., New York, 1927. Price \$6.00. This book is presented so that the layman as well as the chemist can understand the subject treated. From the standpoint of the chemist, food inspection official, attorney and manufacturer, the compilation of food standards and modern data on composition is very thorough. Over 80 tables present data of 70 food products, and in connection with each table comments in non-technical language are made upon the latest legal standard. Legal labeling as presented by such authorities as Federal food inspection "Decisions" and "Service and Regulatory Announcements" of the United States Department of Agriculture is given in many instances. The book covers all fruit products, condimental sauces, all edible oils, cocoa products, malt, sugar and spirit vinegars, spices and maple products. Some attention is given to the buying of vinegars and spices. A compiled appendix on carbonated fruit beverages, pectin technic and mayonnaise manufacture concludes the book.

Corrections.

Since publication of the paper, "Comparison of the Monier-Williams and the A. O. A. C. Methods for the Determination of Sulfurous Acid in Food Products", in the February 15, 1929, number of This Journal, the author has learned that A. Chaston Chapman, London, England, first recommended using hydrogen peroxide for the purpose of distinguishing between sulfurous acid and organic sulfur compounds and that his experiments were carried out in the cold. Therefore he is grateful for this opportunity of giving credit to Mr. Chapman in connection with a statement on p. 121 under the heading, "Discussion of Method".

In Vol. XII, No. 1, p. 38, the quantity of pure copper specified in the second line of the directions for preparing standard thiosulfate solution should be 0.2, not 0.02 as given.

Referees and Associate Referees.

The following associate referees have been appointed since the publication of the list¹.

Arsenic:

W. C. Taber, Food, Drug and Insecticide Adm., San Francisco, Calif.

Boron:

O. F. Krumboltz, Bureau of Chemistry and Soils, Washington, D. C.

Tin:

Urner Liddel, Bureau of Chemistry and Soils, Washington, D. C.

Copper and Zinc:

Reed Walker, Bureau of Chemistry and Soils, Washington, D. C.

Diastatic Value of Flour:

Arnold Johnson, Agricultural Experiment Station, Bozeman, Mont.

Foreign Methods for Testing Flour:

C. H. Bailey, University Farm, St. Paul, Minn.

Organic and Ammoniacal Nitrogen in Air-dried Baked Cereal Products:

S. C. Rowe, Food, Drug and Insecticide Adm., Washington, D. C.

Crude Fiber in Alimentary Pastes and in Air-dried Baked Cereal Products:

W. F. Sterling, Food, Drug and Insecticide Adm., Washington, D. C.

Collecting and Preparing Sample of Alimentary Paste for Analysis:

J. B. Reed, Health Dept., Municipal Bldg., Washington, D. C.

¹ This Journal, 1929, 12: 4.



ANTHONY MCGILL, 1847-1928.

ANTHONY MCGILL

Dr. Anthony McGill was born at Rothesay, Scotland, April 18, 1847. He died in Berkeley, California, December 29, 1928. At the age of ten his family moved to Canada and settled at Hamilton, Ontario, where he received his grammar school education. He graduated from the University of Toronto as B.A. in 1880 and immediately secured a position as assistant on the staff. In 1885 he secured his science degree from Victoria University for his ability as a chemist and scholar. Two years later he was appointed as the first fully qualified chemist in the Food and Drug Laboratories at Ottawa. This marked the beginning of Federal control work in foods, drugs, feeding stuffs and fertilizers in the Dominion of Canada.

The growth of his work was steady and sure. An inspection staff was appointed to visit retailers, wholesalers and manufacturers of products which were under the control of the Adulteration Act. From time to time branch laboratories were opened at Halifax, Montreal, Winnipeg and Vancouver to facilitate the work of these inspectors and make the law easier to enforce. By wise legislation and patient education the percentage of adulteration was reduced from as high as 90 per cent to 5 per cent of the samples analyzed. During this period Dr. McGill was intimately associated with the growth and development of the Association of Official Agricultural Chemists. His name appears as Associate Referee on Cereal Products in 1901, which position he held for several years. He also presented several papers during his membership in the association.

Dr. McGill became Chief Dominion Analyst for the Dominion of Canada in 1907, and he retained this position until his retirement in 1919.

In private life Dr. McGill was a scholar and philosopher. His hobbies were music and photography. His love for nature manifested itself in his study of astronomy and botany. His genial smile and sparkling wit placed his personal relationship on a firm and happy basis.

There is a definite place in the history of chemistry for Dr. McGill. He had a broad vision in the early days when chemistry was but in its infancy, and the advancement of the sciences to their present state have all come, in so far as North America is concerned, within the span of his lifetime.

G. E. GRATTAN.

FIRST DAY.

MONDAY—AFTERNOON SESSION—Continued.

DRUG SECTION.

REPORT ON DRUGS.

By ARTHUR E. PAUL (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Referee*.

Last year the work on six topics was completed, and eight new topics were proposed for study. The total number of associate referees this year was 19, but four were unable to present reports owing to resignations or to other satisfactory causes. The work was carried on with the assistance of an unusually large number of collaborators, to whom the gratitude of the association is due.

While at this time it is recommended in only four cases that the topics be closed, it is felt in all other instances that the respective subjects were distinctly advanced as a result of this year's work. It is also believed that the quality of the reports this year is unusually high.

The researches resulted in recommendations for the tentative adoption of nine proposed methods, and a marked improvement was made in one method which is now tentative. It is also assumed that methods which were made tentative last year will be regularly advanced for adoption as official methods.

It is recommended—

(1) That the following topics be discontinued:

Alcohol in Drugs,
Chloroform and Carbon Tetrachloride,
Pilocarpine in Tablets, and
Sabadilla Assay.

(2) That the following topics be continued:

Laxatives and Bitter Tonics,
Mercurials,
Microchemical Methods for Alkaloids,
Santonin,
Bioassay of Drugs,
Ephedra,
Thymol,
Menthol,
Chlorides and Bromides,
Chenopodium, and
Ether.

(3) That the following subjects be continued as recommended last year by Subcommittee B, since no reports were submitted by the associate referees this year:

Crude Drugs,
Radioactivity in Drugs and Waters,
Terpin Hydrate, and
Fluidextract of Ginger.

(4) That the following new subjects be studied and that an associate referee be appointed in each instance:

Bismuth Compounds in Tablets,
Phenol and Salicylates in Mixtures,
Small Quantities of Iodides in Mixtures,
Chemical Vitamin Methods, and
Phenolsulfonates.

(5) That consideration be given to the following comments relating to the reports submitted by associate referees:

ALCOHOL.

The associate referee studied the determination of methyl alcohol in the presence of ethyl alcohol. The method proposed is, in effect, a modification of the chemical colorimetric procedure studied last year. The results reported are quite satisfactory, and while the associate referee refrains from making a recommendation relative to this method, it is suggested that it be tentatively adopted.

The immersion refractometer method¹ apparently includes certain inaccuracies. The tables are now being revised by one of the authors of the method (Lythgoe), and in due time they will be presented to the association for correction.

The associate referee recommends that the matter of interference by formaldehyde, paraldehyde and isopropyl alcohol be referred to the Referee on Beers, Wines and Distilled Liquors. This recommendation is approved, and this subject may then be considered closed.

CRUDE DRUGS.

Last year's associate referee on this topic devoted his attention to fluidextract of ginger. That preparation can hardly be classified as a crude drug, but in view of its importance, his report is included under that heading. Therefore, the subject of crude drugs must now be considered essentially a new topic. No report was received from the associate referee, because he severed his connections with the Department of Agriculture. It is recommended that this subject be continued.

¹ *Methods of Analysis*, A. O. A. C., 1925, 372.

CHLOROFORM AND CARBON TETRACHLORIDE.

The researches of former Associate Referee Moraw were of much value in developing satisfactory methods for the determination of these important substances. Although they were adopted tentatively, they were considered rather time-consuming and also involved certain features which appeared to require additional study.

The later interesting and exhaustive study, however, has cleared up these uncertainties and resulted in a modified procedure which is materially shorter and in point of accuracy leaves little to be desired.

The recommendations of the associate referee may be restated as follows:

(1) That the method now tentative be replaced by the modified procedure proposed by the associate referee, with official adoption in view.

(2) That this subject be considered closed.

RADIOACTIVITY IN DRUGS AND WATERS.

The program of the associate referee for several years has been so extensive that it necessarily covers a number of years. He has prepared a number of collaborative samples for study, but this year has been unable to bring the work to a point warranting the preparation of a report. Failure to submit a report, therefore, does not indicate that the work on this topic is not making satisfactory progress.

LAXATIVES AND BITTER TONICS.

The associate referee discarded the methods studied by his predecessor because they were not entirely satisfactory. He studied new procedures, which include separation of anthraquinones into active and possibly non-active bodies. He reports that the collaborative results are promising and that they should be further studied, and also makes suggestions that should be of value to his successor.

MERCURIALS.

Last year the associate referee adapted the U. S. P. calomel method to the determination of mercury in calomel tablets. This method was adopted tentatively by this association.

This year he devised a similar adaptation of the U. S. P. mercurous iodide method for the determination of mercury in mercurous iodide tablets. The results reported by collaborators are quite satisfactory, and the associate referee's recommendation for tentative adoption is approved, as are also his further recommendations for a slight change in the calomel tablet method and his suggestion that next year a method be devised for mercuric iodide tablets.

MICROCHEMICAL METHODS FOR ALKALOIDS.

Of the eleven important alkaloids that have been studied, one, namely heroine, is now official and appears in *Methods of Analysis*: four, namely cocaine, codeine, morphine, and strychnine, were made tentative in 1927; two, atropine and pilocarpine, were also studied in 1927, but were not recommended for adoption because of some uncertainties in the results. They were, however, given further attention by the associate referee this year and are now recommended by him for tentative adoption. The four cinchona alkaloids, quinine, quinidine, cinchonine and cinchonidine, were studied this year and, in view of satisfactory collaborative findings, are recommended by the associate referee for tentative adoption.

These recommendations are approved, and it is further recommended that the methods for cocaine, codeine, morphine and strychnine be made official (2d reading).

TERPIN HYDRATE.

Initial consideration of this subject was instituted by the associate referee last year, and a method studied by him proved quite promising. Sufficient work was not done, however, to recommend adoption. The associate referee this year was unable to carry on the investigation. It is now recommended that last year's recommendation of Subcommittee B be repeated this year.

SANTONIN.

This subject has been assigned to associate referees on various occasions, but aside from qualitative tests no work has been accomplished. This year a study was made on santonin mixed with possible interfering substances, four different published methods being used.

Each of the methods specifies weighing the chloroform-soluble material, and all, no doubt, were originally intended as assays for wormseed. As might be expected, the presence of fatty materials in the mixtures rendered all four methods inapplicable. The results on a non-fat mixture also gave results far too low, and the reports submitted show that each of the methods extracts an unsatisfactorily large quantity of chloroform-soluble material from tragacanth and sugar.

In harmony with the associate referee's recommendations, it is believed that the subject should be restudied, that consideration be given to the methods mentioned by Palkin¹, and that, if necessary, an attempt be made to devise a new method.

ETHER.

Last year's associate referee carried on some interesting work on this subject, but in view of the extremely difficult nature of the problem was unable to complete the work. It was therefore reassigned this year.

¹ *This Journal*, 1926, 9: 326.

The present associate referee studied a method, the principle of which is well known, but heretofore it has not been found practicable. In view of the remarkably satisfactory results obtained by him, it seems that he has so arranged the details that they constitute a workable procedure. It is recommended that a collaborative study be carried on during the coming year.

BIOASSAY OF DRUGS.

With the adoption, in 1927, of the cat eye method for mydriatics and myotics, the investigations made up to that time were completed.

Although the associate referee appointed for this topic severed his connections with the U. S. Government, he and Wm. T. McClosky carried on some work and have submitted a report, which is referred to next year's associate referee for attention in addition to any further work or other lines of investigation which he may decide to carry on.

FLUIDEXTRACT OF GINGER.

Some work was done last year by the associate referee, and it was decided to continue the topic. It is regretted that no report was submitted this year, and that it will be necessary to continue the subject for another year.

EPHEDRA.

This crude drug has been known and used by the Chinese for thousands of years, but up to a few years ago it was almost unknown here. Attention was directed to it by the Chinese investigator, K. K. Chen. Since then a great deal of work has been done, and much splendid information may be found in the recent literature.

In view of the present importance of the drug, a satisfactory alkaloidal assay is important, but its formulation is complicated by the unusual properties of the alkaloid ephedrine. Although the work now shows definite progress and the results obtained are promising, the associate referee's conclusions for further study should be approved.

PILOCARPINE IN TABLETS.

This is a new topic in this association, and the results obtained by the collaborators show the method studied to be satisfactory. The recommendation by the associate referee, for its tentative adoption, is therefore approved, and it is further recommended that this topic be now considered closed.

THYMOL.

The work on this new topic, so far as consideration by this association is concerned, is also gratifying. A satisfactory method was perfected by the associate referee, and it is now recommended for tentative adoption.

In harmony with the suggestion made by the associate referee, it is recommended that this topic be continued for another year to include a study of the determination of thymol in mixtures.

MENTHOL.

This is a new subject for which the associate referee utilized a modification of the U. S. P. method for peppermint oil; it appears to have yielded promising results. However, he feels that further study should be devoted to the method, particularly since some of the slightly high results indicate possible difficulty in removing acetic anhydride from the acetylated oil. Some suggestions were made by Leslie Hart. It is recommended that the method be further studied, as suggested by the associate referee, and that the investigation include the determination of menthol in mixtures.

CHLORIDES AND BROMIDES.

The associate referee studied Winkler's method for the determination of bromides in the presence of chlorides, which apparently has much merit. It should, however, be subjected to collaborative study. It would also seem desirable to test the method for applicability to small quantities of bromine in the presence of relatively large quantities of chlorides.

Some work on the determination of mixed halides by potentiometric methods has been done by various investigators, and it is believed that these merit some further attention.

It is recommended—

(1) That the method for bromides in the presence of chlorides, described this year by the associate referee, be subjected to collaborative study.

(2) That the applicability of potentiometric methods be studied.

(3) That the problem of the separation by chemical means of the three halogens—chlorine, bromine and iodine—be studied.

CHENOPODIUM OIL.

Heretofore no actual work has been done on this topic by the A. O. A. C. In view of criticisms of the U. S. P. method, it seemed desirable to have available a method which has the approval of this association. It is considered fortunate, therefore, that a method devised by Paget, which was studied this year, appears to be very promising. Because some of the results reported on pure oils differ from the U. S. P. results, the associate referee suggests that further work is desirable. It is recommended that the titanium trichloride method be further studied, collaboratively, and if found satisfactory that it be rewritten so as to conform as nearly as possible to the general form of the tentative and official methods.

SABADILLA.

This also is a new subject. The literature appears to show wide variation as to alkaloidal content of this seed, the associate referee having found statements which include 0.3 per cent and 5.37 per cent as extremes. He studied collaboratively a modification of the type A, U. S. P. assay, and the results reported are in satisfactory agreement throughout, comparing favorably with results obtained by the associate referee, who used modifications of the procedure. Since he also included in his report a justifiable conclusion that the result obtained represents alkaloids only, it is believed that the recommendation for tentative adoption should be approved.

REPORT ON ALCOHOL IN DRUGS.

By E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.),
Associate Referee.

The associate referee a year ago outlined nine topics for investigation and secured collaborative study upon most of them. The topics outlined were as follows:

The determination of alcohol (1) in the presence of iodine; (2) in oil emulsions; (3) under the conditions which obtain in chloroform liniment; (4) in the presence of 1 per cent of formaldehyde; (5) in the presence of 20 per cent of formaldehyde; (6) in the presence of 3 per cent of paraldehyde; (7) in the presence of 1 per cent of methyl alcohol; (8) in the presence of isopropyl alcohol; and (9) the determination of alcohol in small quantities.

The results obtained where iodine was the only interfering substance were satisfactory, but since the U. S. P. X. gives directions for the determination of alcohol under this condition, no recommendation for adoption of this method by this association was made. The text just cited also gives directions for the determination of alcohol in the presence of volatile oils and in chloroform liniment, and hence no action with respect to adoption of methods for items (2) and (3) is called for except to emphasize the suggestion made by the associate referee last year that the ether extracts of the original solution be very thoroughly washed with saturated salt solution.

No study was made of methods for the estimation of isopropyl alcohol or of alcohol in the presence of that substance. It is understood that the Revision Committee of the U. S. P. has this matter under investigation, but no information was obtained by the associate referee regarding progress of that work. An inquiry made in accordance with the suggestion of the referee last year brought no response.

As for the determination of small quantities of alcohol, so far as these can be determined by the usual methods involving observations of specific gravity and of refraction, there appears to be no need for distilling to a smaller volume than that of the original solution except for quantities considerably less than 0.5 per cent. In such cases the advantages of smaller volumes are obvious, but collaborative study or special methods do not appear to be necessary.

No further work was done upon methods for determining alcohol in the presence of paraldehyde and of formaldehyde. These methods appear to need further investigation before they are submitted again to collaborative study.

The associate referee last year outlined a procedure for the determination of alcohol in mixtures containing relatively small quantities of methyl alcohol, and the results of collaborative trials were fairly satisfactory. The procedure was not recommended for adoption and hence was not stated in a form to be applied to an unknown solution. The method presented this year is basically the same as that proposed last year, but it differs in some points of technic and manipulation which appear to be necessary or advisable in order to make it more generally applicable. It has been used substantially in this form in the laboratory of the present associate referee for several years with satisfactory results.

The method has been published¹.

A few results obtained upon two experimental mixtures prepared according to the following directions will illustrate its applicability:

PREPARATION OF SAMPLE.

Prepare about 500 cc. of an approximately 40 per cent solution of alcohol and transfer a 50 cc. portion to each of six 100 cc. volumetric flasks. (1) Dilute two of these to 100 cc. at 20°C. (2) To two others add 10 cc. of Solution A¹ and dilute the mixture to 100 cc. (3) To the remaining two flasks add 4 cc. of Solution A and dilute to 100 cc. Make all transfers and dilutions at 20°C. and mix each sample thoroughly.

The collaborators were C. E. Shepard and W. T. Mathis, both of the Connecticut Agricultural Experiment Station. To one of them (C. E. S.) the development of the procedure is largely due.

It is evident from the directions that the quantity of ethyl alcohol present in each solution was closely approximate to 20 per cent by volume and that the quantities of methyl alcohol taken were closely approximate to 2.5 and 1.0 per cent, respectively. The recoveries as shown by the results given in the table are very satisfactory.

A single color standard cannot be used for estimating varying amounts of methyl alcohol because the intensity of color does not progress in

¹ *This Journal*, 1929, 12: 49.

Determinations of ethyl and methyl alcohols in experimental mixtures.

(Results expressed as percentage by volume.)

		C. E. SREARD	W. T. MATHIS
Solution 1.	Total alcohol found.....	{ 20 20 20 12	{ 19.88 19.76
Solution 2.	Total alcohols found.....	{ 22 52 22 52	{ 22.24 22.28
	Methyl alcohol found colorimetrically.....	{ 2 50 2 50	{ 2.40 2.40
	Ethyl alcohol, by difference.....	{ 20 02 20 02	{ 19.84 19.88
Solution 3.	Total alcohols found.....	{ 20 92 21 00	{ 20.72 20.80
	Methyl alcohol found colorimetrically....	{ 1 00 1 00	{ 0.95 0.95
	Ethyl alcohol, by difference.....	{ 19 92 20.00	{ 19.77 19.85

direct simple proportion to increasing percentages of methyl alcohol. Thus, the intensity of color produced in a solution containing 2 per cent of methyl alcohol is not twice as great as that produced by a 1 per cent solution but nearly 2.8 times as great. On the other hand, if a 2 per cent solution of methyl alcohol is judged by comparison with a standard containing a higher percentage, the result will indicate a lower percentage of methyl alcohol than is actually present. No doubt a factor could be evolved to meet this situation, but a series of standards seemed the simplest and most direct method of procedure.

At present the estimation of small percentages of methyl alcohol in mixtures with ethyl alcohol by means of the official refractometric table is unreliable. With the revision of this table, which is now in progress, it may be found possible to determine these small amounts with sufficient accuracy. But even so, a colorimetric procedure has the added advantage of furnishing at once both qualitative and quantitative evidence. The results obtained by the method here described appear to be sufficiently satisfactory to warrant adoption of the method as tentative.

There appears to be no need for further consideration of methods for the determination of alcohol in the presence of iodine, of volatile oils, or of the substances as found in chloroform liniment. Likewise further consideration of the determination of small quantities of ethyl alcohol, as contemplated by item 9 in the introduction of this report, may well be abandoned.

It seems advisable to continue study of methods involving formaldehyde, paraldehyde and isopropyl alcohol upon which no progress has been made this year, and to secure some further collaborative study of the procedure described this year for the estimation of alcohol and methyl alcohol when occurring together. Since these problems are conspicuously

those which confront the analyst having to examine alcoholic beverages at the present time, it seems appropriate to refer these topics to the Referee on Beers, Wines and Distilled Liquors.

RECOMMENDATIONS¹.

It is recommended—

(1) That the procedure described for the determination of ethyl alcohol in the presence of methyl alcohol be adopted as a tentative method.

(2) That the associate refereeship on the determination of alcohol in drugs be discontinued.

(3) That the suggestions for further study which are made in this report be referred to the Referee on Beers, Wines and Distilled Liquors for such action as he may deem necessary or advisable.

No report on crude drugs was given by the associate referee.

REPORT ON CHLOROFORM AND CARBON TETRACHLORIDE.

By W. F. KUNKE (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation of last year², investigational work was done to develop a method for the determination of chloroform in mixtures, and further study was made of the tentative method for chloroform and carbon tetrachloride³ to determine (1) the need of the pressure bottle and (2) the necessity of heating to complete the reaction, with the view to working out an equally accurate but simpler procedure. Several other details of the tentative method were also studied. Those proposed by Moraw⁴ for the determination of chloroform in mixtures were not studied. However, a simpler and more accurate method, which does not involve distillation of the chloroform, was developed.

The procedure proposed by Moraw involves distillation of the chloroform and subsequent determination in the distillate by the tentative method. Moraw's results⁵ on a mixture of sirup of white pine bark and chloroform vary from 88.7 to 93.6 per cent, and his results on chloroform in equal parts of alcohol and water vary from 95.1 to 95.75 per cent recovery. Willgerodt⁶ had previously reported that unsatisfactory results were obtained when the chloroform was distilled. He used essentially the same method as that proposed by Moraw later. It is

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 74.

² *This Journal*, 1928, 11: 315.

³ *Ibid.*, 1927, 10: 45.

⁴ *Ibid.*, 358.

⁵ *Ibid.*, 352.

⁶ *Am. J. Pharm.*, 1925, 97: 584.

believed that any quantitative method for the separation of chloroform in mixtures by distillation is open to the criticism of giving inaccurate results.

SAMPLES USED.

Two different samples of chloroform were used. No. 1, containing 99.5 per cent chloroform and 0.5 per cent alcohol by weight, was the chloroform purified by Moraw; it was designated as Chloroform Sample No. 1 and used as a collaborative sample in 1926. No. 2 was found by the tentative method to contain 99.58 per cent chloroform by weight, the average of seven determinations.

The carbon tetrachloride sample was of highest purity and without added preservative; it was repurified by Moraw, designated as Collaborative Sample No. 2 Carbon Tetrachloride—Pure, and used as a collaborative sample in 1926.

All samples were free from chloride, free chlorine and chlorinated decomposition products¹.

In the study of the various details of the tentative method a large number of determinations and experimental weighings were made to secure data on the following uncertain points:

Necessity of—

- 1—Weighing sample without use of weighing bottle.
- 2—Using pressure bottle.
- 3—Ascertaining whether a saturated solution of potassium hydroxide in methyl alcohol is a more active saponification reagent than the alcoholic potassium hydroxide specified.
- 4—Heating to complete the reaction.
- 5—Heating three hours at boiling temperature of water.
- 6—Standing with reagent for 1 hour before heating.
- 7—Shaking the reaction mixture.
- 8—Completing solution of the carbon tetrachloride sample immediately.

WEIGHING OF SAMPLE.

Since the samples for all the determinations reported this year were weighed directly in the saponification flask, this detail will be taken up first.

According to the tentative method, a 0.2–0.5 gram sample is weighed in a ground-glass stoppered weighing bottle of 1–2 cc. capacity. It is desirable to weigh the sample directly in the flask used for the saponification, particularly so when the sample is a mixture such as Compound Sirup of White Pine, N. F., which contains only 0.6 per cent chloroform by volume, and when a larger sample should be taken than could be weighed in a 1–2 cc. weighing bottle. Weighing directly in the saponification flask would give a uniform procedure for all samples.

¹ Tests for Purity, U. S. P. X., p. 106.

Experimental weighings were made of a 1 cc. sample of chloroform weighed directly in a tared rubber-stoppered Erlenmeyer flask of 300 cc. capacity containing 30 cc. of alcoholic potassium hydroxide. This reagent is the same as that used in the tentative method and is made by dissolving 30 grams of potassium hydroxide in 30 cc. of water and adding sufficient methyl alcohol to make 100 cc.

The 1 cc. sample of chloroform was drained from a 1 cc. pipet, which was held just above the top level of the reagent in the flask so as to avoid contact with the reagent. It immediately passed through the reagent layer in a fine stream to the bottom of the flask. The flask and contents were again weighed, and the difference in weight was the weight of the chloroform.

It was found that 20 seconds is a convenient period of time to remove the rubber stopper from the Erlenmeyer flask, drain the chloroform from the 1 cc. pipet, and replace the stopper. The Erlenmeyer flask was opened for periods of 20 seconds, and the losses in weight due to evaporation during ten such periods varied from 1 to 3 mg., or an average loss in weight of 2 mg. per period. This loss is negligible on a basis of a 1.475 gram sample of chloroform, for the correction would be only -0.14 per cent. When a 60-70 cc. pressure bottle is used the loss would be still less, for the surface area of the reagent would be considerably less.

ERLENMEYER FLASK EXPERIMENTS.

An Erlenmeyer flask, tightly stoppered with a rubber stopper or connected to a long spiral reflux condenser with an 18-inch water jacket, was used. For some experiments in which the reflux condenser was used a small quantity of anilin was added to utilize the carbylamine reaction in addition to the saponification. The carbylamine reaction takes place according to the following equation: $\text{CHCl}_3 + \text{CH}_3\text{NH}_2 + 3\text{KOH} = \text{CH}_2\text{NC} + 3\text{KCl} + 3\text{H}_2\text{O}$. Heat was applied only in the experiments requiring the reflux condenser. The boiling point of the reaction mixture was approximately 83°C. when the alcoholic potassium hydroxide, Reagent A, was used. The heat of reaction (30 cc. alcoholic potassium hydroxide, Reagent A, -1 cc. chloroform) warmed up the reaction mixture in the determinations that were left standing at room temperature. The maximum temperature of 50°C. was reached in about 15 minutes; then the mixture cooled off to room temperature (30°C.) in about 1 hour.

Table 1 gives results obtained with a 300 cc. Erlenmeyer flask, tightly closed with a rubber stopper, and either 30 cc. of alcoholic potassium hydroxide, Reagent A, or B and left standing for various periods of time, without heating.

REAGENTS.

Alcoholic potassium hydroxide, Reagent A, is the same as that specified in the tentative method and is made by dissolving 30 grams of potassium hydroxide in 30 cc. of water, cooling and diluting with methyl alcohol to 100 cc.

Alcoholic potassium hydroxide, Reagent B, is made by dissolving 35 grams of potassium hydroxide in sufficient methyl alcohol to make 100 cc. (This is a saturated solution.)

In Experiments 23-24, 5 cc. of soap liniment, U. S. P. X., was added to and thoroughly mixed with Reagent B before the 1 cc. of chloroform sample was added and weighed. The sample-mixture then simulated a 5 cc. sample of chloroform liniment, U. S. P. X., which contains 30 per cent of chloroform by volume.

Weighings of the chloroform were made directly in the Erlenmeyer flask as explained previously under "Weighing of Sample".

TABLE 1.

Results of experiments with stoppered Erlenmeyer flask obtained by associate referee.

EXPERIMENT NO.	CHLOROFORM SAMPLE NO. 2 99.5 PER CENT BY WEIGHT*	REAGENT 30 CC.	REACTION PERIOD (NO HEATING OR SHAKING)	CHLOROFORM FOUND
	grams		hours	per cent by weight
1	1.4720	A	$\frac{1}{4}$ (Lost 0.015 gram.)	55.4
2	1.4720	B	$\frac{1}{4}$	97.1
3	1.4680	A	$\frac{1}{2}$ (Lost 0.001 gram.)	73.2
4	1.4785	A	$\frac{1}{4}$	82.1
5	1.4690	A	1	96.7
6	1.4740	A	1	97.3
7	1.4665	A	1 (Lost 0.0015 gram.)	97.5
8	1.4740	B	1	97.7
9	1.4730	A	3	97.5
10	1.4750	A	3	97.7
11	1.4725	B	3	97.9
12	1.4660	A	6	97.5
13	1.4750	A	6	97.8
14	1.4640	A	18	98.0
15	1.4735	A	18 (Stopper blew out.)	97.1
16	1.4726	B	18	98.4
17	1.4730	B	18	98.1
18	1.4640	A	19	97.5
19	1.4645	A	19	97.7
20	1.4643	A	19	97.9
21	1.4640	A	19	98.1
22	1.4710	A	45 (Refrigerator)	63.9
23	1.4765†	B	$2\frac{1}{2}$ (Lost 0.007 gram.)	96.3
24	1.4525†	B	20 (Lost 0.001 gram.)	97.5

* 99.58 per cent by weight average of experiments Nos. 1-4, Table 3.

† 5 cc. soap liniment mixed with Reagent B before chloroform was added.

Table 2 gives results obtained by using a 300 cc. Erlenmeyer flask with a reflux condenser and 30 cc. of alcoholic potassium hydroxide, Reagent A.

The reaction mixture was first allowed to stand at room temperature, the flask being closed tightly with a rubber stopper, and then it was boiled under a long spiral reflux condenser having a water jacket 18 inches long and the upper opening loosely closed with a rubber stopper. The mixture was gradually cooled to room temperature while still connected with the condenser, and the chloride was determined immediately or allowed to stand at room temperature for varying periods. The determination was made volumetrically by the Volhard method.

Weighings were made directly in the flask as stated under "Weighing of Sample".

TABLE 2.

Results of experiments with Erlenmeyer flask, reflux condenser and Reagent A obtained by associate referee.

EXPERIMENT NO.	CHLOROFORM SAMPLE NO. 2	REACTION PERIOD			CHLOROFORM FOUND
		Room temperature (before heating)	Boiled under reflux (about 83°C.)	Room temperature (after heating)	
	grams	hours	hours	hours	per cent by weight
1	1.4635	$\frac{1}{2}$	$\frac{1}{2}$...	93.8
2	1.4740	$\frac{1}{2}$	$\frac{1}{2}$...	96.0
3	1.4745	$\frac{1}{2}$	$\frac{1}{2}$...	95.9
4	1.4735	26†	1	...	97.8
5	1.4650*	1	2	18	97.4
6	1.4770	3	2	...	97.5
7	1.4672*	18	2	20	98.5
8	1.4720*	21	2‡	...	97.8
9	1.4755*	23	2	16	97.8
10	1.4575*	2	3	...	97.8
11	1.4725	3	3	18	98.5
12	1.4630	18	3‡	...	98.3
13	1.4713	18	3	...	98.2
14	1.4645*	18	3‡	2	97.3
15	1.4785	18	3‡	...	97.7
16	1.4718	24	3	...	98.2
17	1.4730	...	6	18	96.5

* Chloroform Sample No. 1 (99.5 per cent chloroform by weight).

† Loss in weight of flask and contents; Experiment No. 4, 0.003 gram in first 20 hours; No. 11, 2.260 grams in 24 hours; No. 15, 0.009 gram in first 18 hours; No. 16, 0.0035 gram in first 20 hours; No. 17, 1.405 grams in first 6 hours.

‡ With 1 cc. anilin.

DISCUSSION OF RESULTS (TABLES 1-2).

The results of these experiments show that complete decomposition of chloroform or carbon tetrachloride cannot be obtained by using an Erlenmeyer flask, tightly stoppered, and without applied heat, or by using heat and an Erlenmeyer flask under a reflux condenser. Experiments Nos. 1-3-7-23-24, Table 1, and Nos. 4-16, Table 2, show that there was *no appreciable loss in weight* of the mixture during the reaction period. Therefore the low results cannot be attributed to a loss in chloroform, so far as these experiments are concerned.

Experiments Nos. 11 and 17, Table 2, show that there was an appreciable loss in weight of the reaction mixture when boiled under a reflux condenser. Alcoholic potassium hydroxide, Reagent B (non-aqueous), decomposes chloroform so readily that it cannot be used in the determination of chloroform (pure or nearly so) in the quantity used when weighed directly in the flask containing the reagent because the heat of reaction causes the chloroform to boil, which interferes with the weighing.

The results obtained in Experiments Nos. 14, 15, 18-21, inclusive, Table 1, confirm the results reported by Moraw for four determinations with chloroform, 96.98-98.8 per cent, and two determinations, 43.7 and 61.1 per cent, with carbon tetrachloride that was left standing over night unheated.

SUMMARY (TABLES 1-2).

1.—Pressure bottle and heating are required to complete the decomposition of chloroform and carbon tetrachloride. (By comparison with results obtained in Table 3, Experiments 1-10, inclusive; Table 4, Experiments 1-6 and 13-16, inclusive, and Table 5.)

TABLE 3.

Results of experiments with pressure bottle, heat, and Reagent A obtained by associate referee.

EXPERIMENT NO.	CHLOROFORM SAMPLE*	REACTION PERIOD			CHLOROFORM FOUND
		Room temperature (before heating)	Room temperature to 98°-99°C.	Temperature 98°-99°C.	
	grams	minutes	minutes	hours	per cent by weight
1	1.4765	90	45	3	99.20
2	1.4815	75	45	3	99.35
3	1.4720	75	45	3	99.98
4	1.4820	20	45	3	99.75
5	1.4653	120	75	1½	99.52
6	1.4685	90	75	1½	99.47
7	1.4750	90	75	1½	99.78
8	1.4685	90	25	1½	99.95
9	1.4657	75	25	½	100.06
10	1.4770	75	25	½	99.54
			60°-65°C.		
11	1.4783	45	60	...	97.94
12	1.4820	30	60	...	97.76
13	1.4790	20	60	...	97.68
14	1.4780	10	60	...	97.48
			70°-85°C.	85°-90°C.	
15	1.4720	60	15	1½	99.34
16	1.4792	40	15	1½	99.29
17	1.4720	25	15	1½	99.24
18	1.4732	15	15	1½	99.42

* Chloroform Sample No. 2 used in Experiments Nos. 1-7, inclusive. Chloroform Sample No. 1 used in Experiments Nos. 8-18, inclusive, (99.5 per cent by weight).

2.—Alcoholic potassium hydroxide, Reagent B (non-aqueous), is a more active saponification reagent than alcoholic potassium hydroxide, Reagent A, which contains 30 per cent water by volume. Reagent A is used in the tentative method. (Compare Experiment No. 1 with No. 2, Table 1, and Experiment No. 12 with Nos. 13–16, inclusive, Table 4.)

3.—Standing one hour at room temperature, without applied heat, gives just as good results as a longer period. (Compare Experiments Nos. 5–8, inclusive, with Nos. 14–21, inclusive, Table 1.)

4.—Standing at room temperature without applied heat gives just as good results as boiling under a reflux condenser. (Compare Experiments Nos. 5–21, inclusive, Table 1, with Experiments 4–17, inclusive, Table 2.)

5.—Cooling very appreciably retards the rate of saponification of chloroform by alcoholic potassium hydroxide (Experiment No. 22, Table 1).

PRESSURE BOTTLE EXPERIMENTS.

Experiments recorded in Tables 3, 4, and 5 were made to determine whether standing a shorter period of time than 1 hour before heating or 3 hours' heating at 98°–99°C. (boiling temperature of water) is sufficient.

TABLE 4.

Results of determinations of carbon tetrachloride by proposed and tentative† methods and other results obtained by associate referee.*

EXPERIMENT NO.	CARBON TETRACHLORIDE PURE	ALCOHOLIC POTASSIUM HYDROXIDE—PRESSURE BOTTLE	REACTION PERIOD			CHLOROFORM FOUND
			Room temperature (before heating)	Room temperature to 98°–99°C.	Temperature 98°–99°C.	
	<i>grams</i>		<i>hours</i>	<i>minutes</i>		<i>per cent by weight</i>
1	1.5885	Reagent A	1	20	3 hours	100.05
2	1.5890	Reagent A	1	20	3 hours	100.02
3	1.5876	Reagent B	1	20	1 hour	100.15
4	1.5955	Reagent B	1	20	1 hour	100.04
5	1.5890	Reagent B	1	20	1 hour	100.06
6	1.5950	Reagent B	1	20	1 hour	99.97
7	1.5890	Reagent B	1½	20	15 minutes	99.43
8	1.5825	Reagent B	1½	20	15 minutes	99.62
9	1.5907	Reagent B	1½	20	15 minutes	99.11
10	1.5893	Reagent B	1½	20	15 minutes	99.19
11	1.5850	Reagent A	1½	20	23 minutes	99.06
12	1.5935	Reagent A	18	No heat	No heat	53.6
		ERLENMEYER FLASK				
13	1.5860	Reagent B	1	No heat	No heat	86.9
14	1.5870	Reagent B	3	No heat	No heat	94.5
15	1.5835	Reagent B	5	No heat	No heat	94.0
16	1.5900	Reagent B	22	No heat	No heat	93.9

* Experiments Nos. 3–6, inclusive, by proposed method.

† Experiments Nos. 1–2 by tentative method.

Weighings of the chloroform or carbon tetrachloride were made directly in a 60-70 cc. regular pressure bottle containing 30 cc. of alcoholic potassium hydroxide, Reagent A or Reagent B. The tentative method specifies shaking the reaction mixture, but this was not done.

The mixture in the pressure bottle was allowed to stand at room temperature before heating; then, in most cases, the bottle was placed in a water bath at room temperature, and the water was heated to boiling and maintained at that temperature for varying periods of time.

Table 3 gives results obtained with chloroform and Reagent A, heated at different temperatures for varying periods of time.

Table 4 gives results obtained with carbon tetrachloride and either Reagent A or Reagent B, at room temperature, for different periods of time. Additional alcohol was not used. The tentative method directs the use of 15-25 cc. additional alcohol to dissolve the carbon tetrachloride.

Table 5 gives the comparative results (including those obtained by L. Hart, Food, Drug and Insecticide Administration, Chicago, Ill.) by the tentative method and the proposed method. The proposed method has been published¹.

TABLE 5.

Comparative results obtained by tentative method and proposed method†, Reagent A ‡.*

EXPERIMENT NO.	CHLOROFORM SAMPLE NO. 1—99.5 PER CENT BY WEIGHT	REACTION PERIOD			CHLOROFORM FOUND
		Room temperature (before heating)	Room temperature to 98°-99°C.	Temperature 98°-99°C.	
	grams	minutes	minutes	hours	per cent by weight
1	1.4800	60	Not noted	$\frac{1}{4}$	99.78
2	1.4782	60	Not noted	$\frac{1}{4}$	99.92
3	1.4700	30	Not noted	$\frac{1}{4}$	99.69
4	1.4810	30	Not noted	$\frac{1}{4}$	99.80
5	1.4690	90	30	$\frac{1}{4}$	99.63
6	1.4737	75	30	$\frac{1}{4}$	99.60
7	1.4753	75	30	$\frac{1}{4}$	99.82
8	1.4740	75	20	$\frac{1}{4}$	99.37
9	1.4770	75	20	$\frac{1}{4}$	99.50
10	1.4725	40	20	$\frac{1}{4}$	99.34
11	1.4755	20	20	$\frac{1}{4}$	99.34
12	1.4823	60	90	3	99.27
13	1.4770	105	90	3	99.38
14	1.4810	90	90	3	99.75
15	1.4790	75	90	3	99.79

NOTE.—Experiments Nos. 1-4 made by L. Hart, Nos. 5-15 by associate referee.

* Experiments Nos. 12-15, inclusive, by tentative method, but without shaking of reaction mixture.

† Experiments Nos. 1-11, inclusive, by proposed method.

‡ Dissolve 30 grams of KOH in 30 cc. of water and add sufficient methyl alcohol to make 100 cc.

SUMMARY (TABLES 3, 4, AND 5).

Shaking of the reaction mixture is not necessary.

Table 3 (Chloroform).—

¹ *This Journal*, 1929, 12: 50.

1.—A temperature of 60°–65°C. is not high enough to decompose chloroform completely (when heated for 1 hour after standing at room temperature for 10–45 minutes).

2.—A temperature of 85°–90°C. gives good results, which confirm the results obtained by Moraw. This temperature is so near the temperature of boiling water that the latter is favored, because it is more convenient and practical.

Table 4 (Carbon Tetrachloride).—

1.—Reagent B is a more active saponification reagent than Reagent A.

2.—Heating with Reagent B for 1 hour at 98°–99°C. gives just as good results as heating with Reagent A for 3 hours.

3.—Complete solution of the carbon tetrachloride sample is not necessary.

Table 5 (Chloroform).—

1.—Standing for 30 minutes at room temperature before heating gives just as good results as standing for 1 hour, the heating being the same.

2.—Heating for 15 minutes at 98°–99°C. gives just as good results as heating for 3 hours, if the mixture is allowed to stand at room temperature from 15 to 30 minutes before heating.

PROPOSED METHOD FOR CHLOROFORM IN MIXTURES.

Table 6 gives results (including collaborative results by L. Hart) obtained by the proposed method for the determination of chloroform in mixtures. For this study it was thought best *not* to make determinations of chloroform with samples of chloroform mixtures, but to add certain mixtures to the alcoholic potassium hydroxide in the pressure bottle, thoroughly mix, and then add the chloroform. By this procedure it is believed the quantity of chloroform in the pressure bottle is more accurately known than if a chloroform mixture sample were used, particularly in the case of a liniment or cough mixture containing chloroform. Compound Sirup of White Pine, N. F., without chloroform and Soap Liniment, U. S. P. X, were prepared. Compound Sirup of White Pine, N. F. consists essentially of a saturated solution of sugar in water, plant extractives, 17.5 per cent alcohol, 10 per cent glycerin, and 0.6 per cent chloroform by volume. Chloroform Liniment, U. S. P. X, is prepared by mixing soap liniment and chloroform and consists essentially of a hydroalcoholic solution of soap, camphor, oil of rosemary, and 30 per cent chloroform by volume.

In Experiments Nos. 1–14, inclusive, 5 cc. of soap liniment was thoroughly mixed with the 30 cc. of alcoholic potassium hydroxide, Reagent A, in a 60–70 cc. pressure bottle, by gentle swirling, and then the 1 cc. chloroform sample was weighed directly into the bottle. In Experiments Nos. 15–20, inclusive, 10 cc. of compound sirup of white pine, as prepared without the chloroform, was similarly mixed with the 30 cc.

alcoholic potassium hydroxide, Reagent B, in the pressure bottle. To this mixture was then added exactly 1 cc. of a chloroform-alcohol solution containing 14.739 grams of chloroform in 100 cc., or the equivalent of 0.1474 gram of chloroform. The chloroform-alcohol solution was prepared as follows:

Drain 10 cc. of chloroform sample No. 1 from a 10 cc. pipet into a weighed 100 cc. volumetric flask containing about 25 cc. of alcohol, holding the pipet just above the alcohol and avoiding contact with it. Stopper the flask tightly and reweigh. The difference in weight is the weight of the chloroform. Add about 25 cc. of alcohol, mix the chloroform and alcohol thoroughly by swirling, and after cooling to room temperature add enough alcohol to make 100 cc.

TABLE 6.

Chloroform in mixtures determined by proposed method.

EXPERIMENT NO.	CHLOROFORM SAMPLE NO. 1—99.5 PER CENT BY WEIGHT	REACTION PERIOD			CHLOROFORM FOUND
		Room temperature	Room temperature to 98°-99°C.	Temperature 98°-99°C.	
	grams	hours	minutes	minutes	per cent by weight
1	1.4804*	1	Not noted	15	100.1
2	1.4800	1	Not noted	15	99.87
3	1.4850	1½	30	15	99.13
4	1.4860	1½	30	15	98.96
5	1.4860	1½	30	15	99.00
6	1.4775	1	30	15	99.27
7	1.4700	1½		hours	
8	1.4700	1½	40	3	99.52
9	1.4675	1	40	3	99.58
10	1.4715	1	40	3	99.69
11	1.4730	1½	45	3	99.42
12	1.4830	1½	45	3	99.65
13	1.4815	1½	45	3	99.68
14	1.4800	1	45	3	99.62
15	0.1474†	minutes			99.56
16	0.1474†	25	20	1	99.93
17	0.1474†	25	20	1	99.39
18	0.1474†	25	20	1	99.66
19	0.1474†	25	20	1	99.47
20	0.1474†	25	20	1	99.26

NOTE.—Experiments Nos. 1 and 2 were made by L. Hart; all others were made by the associate referee.

* In Experiments Nos. 1-14, inclusive, 5 cc. of soap liniment was previously mixed with 30 cc. of alcoholic potassium hydroxide, Reagent A, in the pressure bottle.

† In Experiments Nos. 15-20, inclusive, 10 cc. compound sirup of white pine, without chloroform, as previously mixed with 30 cc. of alcoholic potassium hydroxide, Reagent B, in the pressure bottle.

DISCUSSION OF RESULTS (TABLE 6).

Hart obtained good results on the two determinations of chloroform in the chloroform-soap liniment mixture with 15 minutes' heating at 98°-99°C. The associate referee obtained better results with 3 hours' heating than with 15 minutes' heating. It is believed that heating

1 hour at 98°–99°C. is sufficient for complete saponification of the chloroform.

Chloroform in chloroform liniment, compound sirup of white pine and similar mixtures can be readily and accurately determined, without distillation of the chloroform, by direct treatment with alcoholic potassium hydroxide in a pressure bottle by the same procedure as for chloroform (pure or nearly so), except that the reaction mixture is heated for 1 hour at 98°–99°C. In the case of compound sirup of white pine, alcoholic potassium hydroxide, Reagent B, is used, and after the saponification of the chloroform the mixture is evaporated, the residue charred and the chloride determined.

COMMENTS ON THE USE OF THE PRESSURE BOTTLE.

For a total of 64 determinations in which there were used the four regular pressure bottles with the same rubber gaskets that Moraw used in 1926, only one gasket was changed. After heating, the bottle should not be allowed to stand closed overnight, because the rubber gasket is likely to stick to the bottle and be spoiled when the bottle is opened. The gasket should fit tightly around the nipple of the porcelain stopper. About 98 per cent by weight of the chloroform (pure) in 1 cc. of chloroform (99.5 per cent by weight) is saponified in 1 hour by 30 cc. alcoholic potassium hydroxide at room temperature without shaking. Consequently, only 2 per cent of the chloroform remains to be saponified with the aid of heat. With alcoholic potassium hydroxide, Reagent A, having a boiling point at atmospheric pressure of about 83°C., and only 2 per cent of the chloroform remaining, it is believed that the pressure exerted by the reaction mixture in the closed bottle when heated to 98°–99°C. is not great.

The ordinary water bath is a convenient place for heating, and four or more determinations can be run at the same time. It is recommended that each bottle be placed in a wire basket and that an empty tin can be inverted over the top of the bottle and the whole covered with towels. No trouble was experienced with the pressure bottle by the associate referee. The need for using it was questioned by some collaborators¹.

COMMENTS ON THE TENTATIVE METHOD.

1.—Nitric acid reagent need *not* be “free from lower oxides” of nitrogen. Ordinary laboratory nitric acid is a satisfactory reagent. It was used in all the determinations reported this year.

2.—*Complete solution* of the carbon tetrachloride sample is *not necessary*, therefore no “additional 15–25 cc. alcohol to the reagent already in the bottle to insure complete solution of the sample” need be added. Additional alcohol was not used in any determination reported this year.

¹ *This Journal*, 1927, 10: 355.

3.—The *sample and alcoholic potassium hydroxide need not be thoroughly mixed and occasionally shaken for 1 hour*. In no determination reported this year was the reaction mixture shaken.

4.—*Heating at 98°–99°C. for 3 hours is not necessary.*

(a) Heating for 15 minutes in case of chloroform is sufficient. (Table 5, Experiments 1–11, inclusive.)

(b) Heating for 1 hour in case of chloroform mixtures and carbon tetrachloride is sufficient. (Table 4, Experiments 3–6, inclusive; Table 6, Experiments 1–6, inclusive.)

5.—*Alcohol need not be evaporated* before acidifying with nitric acid preliminary to determining the chloride by the Volhard method. The alcohol was not evaporated in any determination reported this year.

CONCLUSIONS.

(1) As compared with the tentative method, a simpler and equally accurate procedure for the determination of chloroform and carbon tetrachloride has been worked out.

(2) A new method for chloroform and carbon tetrachloride in mixtures, without distillation, has been developed.

(3) If the sample is a preparation like Chloroform Liniment, U. S. P. X, almost the same procedure is used as for chloroform. If the chloroform mixture is sirupy, like Compound Sirup of White Pine, N. F., the reaction mixture, after the saponification of the chloroform, is evaporated and the residue charred before determining the chloride. It may be remarked that while the proposed method seems long, in its operation it is fairly rapid. The time required for chloroform is about 2 hours; for carbon tetrachloride or chloroform liniment, U. S. P. X, about 3 hours; and for Compound Sirup of White Pine, N. F., about 4 hours.

(4) Weighing of chloroform or carbon tetrachloride, or their mixtures, directly under the reagent in a 60–70 cc. pressure bottle is a simple, satisfactory and accurate procedure.

(5) Pressure bottle and heat are required for complete saponification of chloroform or carbon tetrachloride.

(6) Alcoholic potassium hydroxide, Reagent B (non-aqueous), is a more active saponification reagent than alcoholic potassium hydroxide, Reagent A, which contains 30 per cent water by volume.

(7) Chloroform and carbon tetrachloride cannot be completely saponified by alcoholic potassium hydroxide, Reagent A or B, at room temperature within a reasonable length of time.

(8) Shaking of the chloroform or carbon tetrachloride with the reagent is not necessary. In case foreign material is present, as would be the case when chloroform or carbon tetrachloride is determined in a mixture, gentle swirling of the mixture with the reagent is advisable.

(9) Additional alcohol to insure complete solution of either chloroform or carbon tetrachloride is not necessary.

(10) Cooling of the saponification reagent-chloroform mixture materially reduces the rate of saponification of chloroform.

(11) Chloroform is more readily saponified than carbon tetrachloride by alcoholic potassium hydroxide.

(12) Potassium hydroxide is considerably more soluble than sodium hydroxide in methyl alcohol.

(13) Chloroform containing 0.5 per cent alcohol by weight does not deteriorate when kept in an amber glass bottle, closed with a cork stopper, and stored in a cupboard at room temperature for 2 years. (The bottle was only partially filled.)

(14) The reagents, solvents, soap liniment, U. S. P. X, and Compound Sirup of White Pine, N. F., used did not contain chloride.

RECOMMENDATIONS¹.

It is recommended—

(1) That the present tentative method² for the determination of chloroform and carbon tetrachloride be deleted.

(2) That the methods formulated by the associate referee for the determination of chloroform and carbon tetrachloride and for the determination of chloroform or carbon tetrachloride in mixtures be adopted as a tentative method. This method has been published³.

(3) That the proposed methods be submitted for further collaborative study next year with the view to adopting them as official methods.

No report on radioactivity in drugs and water was given by the associate referee.

REPORT ON LAXATIVES AND BITTER TONICS.

By E. O. EATON⁴ (U. S. Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

No active work has been done on this problem for several years. Work done by the former associate referee, H. C. Fuller, and collaborators indicates that neither the gravimetric nor the colorimetric methods proposed are entirely satisfactory.

Emodin is not the only active ingredient in cascara preparations. It was shown in 1922⁵ that some commercial preparations of merit are

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 74.

² *This Journal*, 1927, 10: 45.

³ *Ibid.*, 1929, 12: 50.

⁴ Presented by W. F. Kunke.

⁵ *J. Am. Pharm. Assoc.*, 1922, 11: 21.

practically free of it. There is some disagreement among pharmacologists as to its laxative properties, but it would seem that this substance alone cannot be relied upon to produce a cascara effect.

The assay of emodin-bearing drugs has been under discussion for several years by this association; therefore no further reference to previous work will be made.

In the method proposed an attempt is made (1) to liberate the acidic principles combined as salts, (2) to extract by chloroform, with a minimum of heat, from a solution of low hydrogen-ion concentration both the free and salt-occurring hydroxyanthraquinones, and (3) to separate the possibly non-therapeutic bodies from those thought to be therapeutically active. In the second portion of the method the use of a mineral acid in essential concentration, aided by heat, hydrolyzes the glucoside present. It also reacts with any resinous-like bodies present to liberate chloroform-soluble substances. Treatment with Caro's reagent¹ shows the resinous bodies to be of hydroxyanthraquinone origin. The procedure employed removes the acidic hydroxyanthraquinones, as liberated, from further contact with the more concentrated mineral acid, which under certain conditions appears to induce the destruction of the anthraquinones.

METHOD.

Introduce 5 cc. of a cascara preparation and 20 cc. of water into a type C continuous extraction apparatus² which has been previously charged with chloroform and follow with 1 cc. of a 1 per cent solution of acetic acid. (A long-stemmed funnel which fits loosely in the upper end of the inner tube will prevent spattering.) Connect with the condenser and reflux by applying heat at the side of the flask, which should carry about 100 cc. of chloroform. Continue the extraction for several hours, depending on the rate of distillation, until the solvent in the apparatus is practically colorless, as shown by a background of white paper. Disconnect the flask. (Note:—Do not discard the contents of the apparatus. See further directions.) Transfer the chloroform to a separatory funnel. If any chloroform-insoluble substances are present in the flask, wash them out, by the aid of a policeman, with a normal solution of sodium hydroxide which has been saturated with salt (Reagent A), and add this solution to the immiscible solvent in the separatory funnel. Now shake thoroughly for several minutes and allow to separate. Repeat this operation, using fresh portions of Reagent A, until all the alkali-soluble substances are in the aqueous layer. To the combined aqueous solution add 25 cc. of chloroform, shake thoroughly, and discard the chloroform. To the alkaline aqueous solution, add slowly concentrated hydrochloric acid to a decided acid reaction. Shake out to completion with chloroform and wash the combined chloroform with 5 cc. of water. Collect the chloroform, after passing it through a plug of cotton wetted with chloroform, in a weighed beaker or evaporating dish. Evaporate the solvent on the steam bath. Dry at 100°C. for 2 hours. Cool and weigh.

Weight $\times 20$ = grams per 100 cc. of chloroform-soluble extractives from the acetic acid digestion. (Including free and salt hydroxyanthraquinones—possibly non-therapeutic.)

To the original apparatus connect a side flask carrying about 100 cc. of fresh chloroform. Add to the inner tube slowly 5 cc. of concentrated sulfuric acid drop by drop, by

¹ *Arch. Pharm.*, 1919, 257: 229; *J. Am. Pharm. Assoc.*, 1923, 12: 695.

² *Ind. Eng. Chem.*, 1925, 17: 612.

means of a pipet. Connect with the condenser and reflux for several hours until the chloroform in the apparatus is practically colorless, as shown by a background of white paper. Disconnect the side flask, extract the alkali-soluble substances, and follow by the chloroform extraction from the acid solution as outlined in the first part of the method following "Note * * *".

Weight $\times 20$ = grams per 100 cc. of chloroform-soluble extractives from the sulfuric acid digestion. (Including probably substances split off from glucosidal and resinous hydroxyanthraquinone bodies—one of the end-products of acid digestion from the therapeutically active substances, no doubt.)

Two samples of liquid cascara preparations were submitted to collaborators. No. 1 was a commercial product, labeled "U. S. P. Fluid-extract of Cascara", and No. 2 was a commercial product of a type similar to aromatic cascara. The collaborators reported as follows:

ANALYST	NO. I		NO. II	
	Free (acetic)	Combined (sulfuric)	Free (acetic)	Combined (sulfuric)
	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>
E. O. E.	0.48	1.06	0.20	1.5
	0.48	1.02	0.22	1.4
	0.50	1.04		
A. W. H.	0.63	1.14	0.44	1.288
R. S. R.	0.62	1.26	0.3	1.96
	0.58	1.08	0.31	1.87
			0.26	
E. H. G.	0.786	1.08	0.288	1.02
	(Note comment)			
	0.790	1.00	0.296	0.95
L. H.	0.572	1.10	0.221	1.60
	0.520	1.02	0.260	1.51
	0.600	1.15		

The collaborators commented as follows:

A. W. Hanson.—It would seem that the addition of strong sulfuric acid is undesirable inasmuch as it generates a considerable amount of heat * * * resulting in considerable charring of the material * * *.

R. S. Roe.—In some instances 2 hours' drying did not seem sufficient to secure constant weight. So far as manipulation is concerned, the method seems to be satisfactory.

E. H. Grant.—Several years ago, when I was with a commercial firm, several manufacturers investigated methods for determining oxyanthraquinone bodies and examined quite a number of samples. We used methods essentially the same as yours, determining both the free emodin and that combined as glucosides. We came to the conclusion that the percentage of these bodies present was no criterion of the therapeutic value of cascara preparations. I think that you are wasting your energies submitting anything for collaborative work unless you can first prove that results obtained by at least one analyst, using the proposed method, really measure the therapeutic value of the medicine. * * *.

* * * I extracted 10 cc. of the fluidextract by the method which you submitted, then washed out the liquid from the thimble, evaporated off the chloroform, neutralized with calcium carbonate, filtered, and drank the equivalent of about 6 or 7 cc. of the original fluidextract. This liquid should be inert, but I got a slight action. This is only one experiment, made in mid-summer when almost anything can cause a slight purgation, but it would suggest that you are probably not getting all the active substances. * * *. Charring occurs * * *.

Leslie Hart.—Two hours is apparently not sufficient time for drying, particularly from sulfuric acid solution.

After further extraction with amyl alcohol and a study of the nature of the extractives, the associate referee arrived at the conclusion that these exhausted materials were physiologically inert. He also swallowed some of the neutralized exhausted matter, which proved to be practically inactive.

Several cascara barks of known age were also assayed by the associate referee by the following procedure:

Grind the air-dried bark and pass through a No. 20 sieve. Macerate 25 grams with 125 cc. of boiling water, stir, cover, and place on the steam bath for 2 hours. Transfer to a small glass percolator with a capillary outlet and pack in such a manner that only slow percolation is permitted. Percolate and add boiling water occasionally until the percolate is a constant pale yellow (about 500 cc. is necessary). Evaporate to about 75 cc. Cool, transfer to a 100 cc. volumetric flask, add 0.5 cc. of chloroform, and make up to volume. Shake well. For assay transfer 20 cc., representing 5 grams of bark, to the continuous extractive apparatus and proceed as above.

The following results were obtained:

AGE OF BARK	FREE ACID (ACETIC)	COMBINED ACID (SULFURIC)
years	per cent by weight	per cent by weight
8	1.34	2.40
6	0.98	1.67
3	0.94	1.94
1	{ 0.34	{ 2.86
	{ 0.33	{ 2.68

The several factors that may influence the content of the bark include soil and weather conditions of growth, part of tree from which bark was produced, manner of curing, and conditions of holding.

CONCLUSIONS.

These methods are encouraging. The variation of results among analysts is largely, it is believed, a matter of interpretation, as they check closely. Further work should be done¹.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 74.

REPORT ON MERCURIALS.

By R. S. ROE (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The work on mercurials this year was concerned largely with a study of mercurous iodide in tablets. Some preliminary work was done on mercuric iodide, but sufficient time was not available for its completion and for the collaborative study. This report, therefore, will review the work on mercurous iodide.

Last year the modified U. S. P. iodine method was adopted as the tentative method for the determination of calomel in tablets¹. This method, with minor changes, has been found satisfactory for mercurous iodide tablets.

SAMPLE FOR COLLABORATIVE STUDY.

The sample for collaborative study was prepared by thoroughly triturating in a mortar the following ingredients: 43 grams of mercurous iodide, 131 grams of lactose, 20 grams of starch, and 6 grams of talc. The mercurous iodide used in the preparation assayed 99.4 per cent. The collaborative sample, then, contained 21.37 per cent of mercurous iodide.

The method has been published².

TABLE 1.
Results reported by collaborators.

	MERCUROUS IODIDE	
	per cent	AVERAGE per cent
E. O. Eaton	21.4	21.65
	21.9	
L. Hart	21.54	21.50
	21.49	
	21.51	
	21.44	
F. L. Elliott	21.46	21.52
	21.57	
E. H. Grant	21.62	21.62
	21.62	
F. C. Synkovich	21.26	21.38
	21.35	
	21.52	
	21.96	
A. W. Hanson	22.07	21.87
	21.59	
	21.59	
H. Wales	20.88	20.82
	20.75	
W. F. Kunke	21.35	21.44
	21.60	
	21.50	
	21.30	
L. E. Warren	21.5	21.65
	21.8	

¹ *This Journal*, 1928, 11: 51.

² *Ibid.*, 1929, 12: 52.

Collaborators were instructed to use about 1.5 grams of the prepared sample and to report results as percentage of mercurous iodide.

COMMENTS OF COLLABORATORS.

E. O. Eaton.—The method is sound and gives good results. It is not exactly clear in some details. A definite time should be stated for the solution of the soluble filler. The "note" should be included in the method, as it presumably does no harm to have alumina cream present, and its use would preclude reassay when it is necessary to use it.

A. W. Hanson.—The method seems to be practicable.

H. Wales.—No Caldwell crucibles were available, and filtration was made through paper as in the Contact Committee method. My past experience with this method has shown that either asbestos or a sufficiently fine grade of filter paper may be used with equally good results.

W. F. Kunze.—No trouble was had with the mercurous iodide passing through the asbestos mat. The method appears to work smoothly and gives good check results.

L. E. Warren.—It may be of interest to know that at least one pharmaceutical manufacturer employing this method for the assay of mercurous iodide and calomel preparations uses 0.2 *N* iodine instead of 0.1 *N* iodine in order to shorten the time of the assay. Using 0.25 *N* iodine, I have found 21.8 per cent and 21.8 per cent of mercurous iodide, allowing 30 minutes to digest. I tried two assays with 15 minutes' standing and obtained 19.9 per cent and 19.6 per cent. Evidently 15 minutes is not enough.

The results reported by the collaborators on the control sample indicate that this method is satisfactory for the assay of mercurous iodide in tablets. The use of an asbestos mat on a Caldwell crucible, with a precaution to use a few drops of alumina cream when necessary, seems to be an improvement over the use of filter paper, because many commercial tablets are difficult to filter through paper without loss.

CALOMEL TABLETS ASSAYED.

A sample of commercial calomel tablets was also assayed, the asbestos mat being used in place of the filter paper specified in the tentative method for the determination of calomel in tablets; after removal of the soluble fillers, the mixture was washed with alcohol and ether. The results are indicated in Table 2 in comparison with results obtained by the tentative method.

TABLE 2.

Assay of commercial calomel tablets (1 grain).

TENTATIVE METHOD	MODIFIED PROCEDURE
gram	gram
0.062	0.064
0.063	0.065

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for the determination of mercurous iodide in tablets, as described, be adopted as a tentative method.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 74.

(2) That the tentative method for the determination of calomel in tablets be amended as to minor details by replacing the section, "Filter through a small filter paper. Wash well with water and return the paper and insoluble material upon it to the flask", with the statement, "decant with the aid of suction through a tightly packed asbestos mat on the removable perforated plate of a Caldwell crucible. Wash once with water by decantation, then successively with alcohol and ether. Transfer the removable plate holding the asbestos mat and insoluble material to the original flask. Wash into the flask any insoluble material adhering to the sides of the crucible;"

(3) That the work on mercurials be continued to complete the study of mercuric iodide in tablets and to study methods for the separation of mercurials from possible interfering substances.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS.

By C. K. GLYCART¹ (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendations made at the last meeting, the work on microchemical methods for alkaloids was continued.

In 1926 and 1927 cocaine, codeine, heroine, morphine, strychnine, atropine, and pilocarpine were submitted for collaborative study. This year the four principal alkaloids of cinchona, quinine, quinidine, cinchonine, and cinchonidine were added to the work.

Directions and descriptions for the identification of the alkaloids, four control specimens consisting of U. S. P. quality of quinine sulfate, quinidine sulfate, cinchonine sulfate, cinchonidine sulfate, and four samples for identification were submitted to the collaborators.

No. 1 consisted of a powdered mixture of cinchonidine sulfate and milk sugar; No. 2 consisted of a 1 cc. ampul containing quinine dihydrochloride in solution; No. 3 consisted of a powdered mixture of cinchonine sulfate and milk sugar; and No. 4 was a solution of quinidine sulfate.

In addition to the microchemical methods, a tabulation showing data pertaining to the specific rotation, solubility in ether, and reaction to the thalleoquin test for the four alkaloids of cinchona was given as an aid in the identification of mixtures of cinchona alkaloids.

The method and the identification tables have been published².

The results and comments of the collaborators are as follows:

H. McCausland, Abbott Laboratories, North Chicago, Ill.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

H. O. Moraw, Swan-Myers Co., Indianapolis, Ind.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

¹ Presented by R. S. Roe.

² *This Journal*, 1929, 12: 53.

Some difficulty was experienced in forming the crystals with potassium iodide and quinidine. It was observed that while they are triangular they might also be described as triangular divided into two halves, one opaque and bounded by a saw-tooth outer edge, and the other transparent with smooth outer edge. The descriptions of all of them might be in greater detail, but when controls are used together with unknowns there may be no necessity for more detail. As a whole, I found the descriptions satisfactory and identifications fairly simple.

Albin Stitt, United Drug Co., Boston, Mass.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

The reactions worked very well, provided as little acid as possible is used to bring the alkaloids into solution. This is especially important in the case of quinidine. An excess of reagent should not be used, otherwise the reagent itself tends to crystallize out and confuse the identification.

R. S. Roe, Food, Drug and Insecticide Administration, Chicago, Ill.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

As applied to these samples the method seems to afford a very satisfactory means of identifying these alkaloids.

Work at this laboratory on products containing a mixture of cinchona alkaloids indicates that it is generally necessary to effect at least a partial separation of the alkaloids to obtain satisfactory results. It may be well to have some collaborative work done on unknown mixtures of these alkaloids.

F. C. Synkovich, Food, Drug and Insecticide Administration, Chicago, Ill.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

W. J. McCarthy, Food, Drug and Insecticide Administration, Cincinnati, Ohio.—No. 1, cinchonidine sulfate; No. 2, quinine sulfate; No. 3, cinchonine sulfate; No. 4, quinidine sulfate.

The study of the crystal formation of the above samples has been very interesting inasmuch as our previous microscopical work has been confined chiefly to food products.

H. R. Watkins and H. Wales, Food, Drug and Insecticide Administration, Washington, D. C.—No. 1, cinchonidine; No. 2, quinine; No. 3, cinchonine; No. 4, quinidine.

When treated with sodium phosphate cinchonine yields immediately a large number of very small, round, possibly burr-shaped, crystals. These crystals are not formed with any of the other above-mentioned alkaloids; the quinine precipitate is formed quite slowly and is of an entirely different shape. We believe that this fact should be included in the characteristic tests.

J. Orion Page, Food, Drug and Insecticide Administration, New York, N. Y.—No. 1, cinchonidine sulfate; No. 2, quinine sulfate; No. 3, cinchonine sulfate; No. 4, cinchonidine sulfate.

The precipitate of Sample 4 with sodium benzoate was not characteristic of cinchonidine.

In the cases of quinine sulfate and quinidine sulfate controls, I found that the addition of one drop of 95 per cent alcohol and 1 drop of distilled water readily dissolved 1 mg. of alkaloid and did not change the character of the precipitate formed.

N. E. Freeman, Food, Drug and Insecticide Administration, Kansas City, Mo.—First report: No. 1, cinchonidine; No. 2, quinine; No. 4, quinidine. No. 3 could not be identified as being any one of the four alkaloids under consideration. The sodium carbonate precipitate seemed to be the same as that obtained with the known sample of cinchonine. The sodium phosphate precipitate formed by this sample appeared to be the most characteristic given by any of the five reagents used. These crystals were small and dark and apparently generally spherical with a roughened surface, presenting much the appearance of a chestnut burr.

Second report: This time I was able to find and identify the rosettes of needles which are described in connection with the sodium carbonate precipitate of cinchonine.

I still believe that the sodium phosphate precipitate of this sample is extremely characteristic. These crystals might possibly be used in connection with the sodium carbonate precipitate as a confirmatory test. It is my opinion, however, that in the case of cinchonine some directions should be given as to the care necessary in getting this precipitate to form slowly, as others might have the same difficulty that I had.

DISCUSSION.

Eight of the ten collaborators made positive identifications of the four unknown alkaloids. One failed to identify quinidine, and one made an inconclusive identification of cinchonine with sodium carbonate reagent in his first report. However, on rechecking the test, he obtained the correct result.

The procedure, modified by the suggestions of Watkins, Wales and Freeman, was repeated by the associate referee, who obtained results that confirmed the value of these suggestions. Accordingly, the descriptions for the identification were amended to include the test specifying sodium phosphate reagent for cinchonine.

On the whole, the results are considered satisfactory.

No further collaborative work was done on atropine and pilocarpine, but the associate referee tested several methods by using authentic specimens of atropine and pilocarpine salts and obtained satisfactory results by the test as given last year¹.

RECOMMENDATIONS².

It is recommended—

(1) That the microchemical tests and descriptions for quinine, quinidine, cinchonine, and cinchonidine be adopted as tentative.

(2) That the microchemical tests for atropine and pilocarpine be adopted as tentative.

(3) That other important alkaloids be further studied with the view to including them in a scheme for identification by microchemical methods.

No report on terpin hydrate was given by the associate referee.

REPORT ON SANTONIN.

By H. M. BURLAGE³ (Laboratory of the Oregon State Board of Pharmacy, School of Pharmacy, Oregon State Agricultural College, Corvallis, Ore.), *Associate Referee*.

Inasmuch as returns on methods were late, the associate referee will make no attempt to present a bibliographic study of santonin and its

¹ *This Journal*, 1928, 11: 354.

² For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 73.

³ Presented by R. S. Roe.

methods of assay. Such a study will be postponed for inclusion in a more comprehensive review of the subject.

Owing to the excessive price of santonin, it was decided (1) to limit the number of collaborators to those who have had some experience along this particular line, namely: L. E. Warren, Drug Research Unit, U. S. Food, Drug and Insecticide Administration; E. O. Ewing, formerly assistant chief chemist, United Drug Company, Boston, Mass.; J. C. Kimmel, an advanced student in the School of Pharmacy, Oregon State College; and the associate referee; (2) to study only those methods already outlined in literature rather than a development of new methods; and (3) to prepare samples of two types of mixtures commonly met with: (a) non-fatty mixtures and (b) fatty mixtures.

It is regretted that the results as a whole are disappointing. This condition, however, is due to the impracticableness and inaccuracy of the existing methods rather than to a lack of effort or of cooperation on the part of the collaborators. None of the methods selected for the assay of fatty santonin mixtures was of value, and the results obtained were so far at variance that they are not included in this report.

PREPARATION OF SAMPLES.

Sample A¹:

	<i>grams</i>
Santonin.....	3
Tragacanth.....	3
Sucrose.....	96

The mixture was passed through an 80-mesh sieve to insure uniform mixture. Four grams of sample was used.

In order to determine the extent to which tragacanth and sucrose, as excipients of the mixture, are soluble in the solvent used, the following sample was prepared:

Sample AA:

	<i>grams</i>
Tragacanth.....	3
Sucrose.....	96

This mixture was passed through an 80-mesh sieve to insure uniformity of sample and 3.8 grams was used for each determination.

Sample B (A fatty mixture)²:

Santonin, in fine powder.....	3	grams
Gluside.....	0.06	gram
Tincture of vanilla.....	1.50	cc.
Cocoa, in fine powder.....	6.00	grams
Sucrose, in fine powder.....	21.00	grams

The sample was passed through an 80-mesh sieve. Samples of 1.2 grams were used in the determinations.

¹ U. S. Pharmacopeia VIII, p. 489.

² National Formulary, 5th ed., p. 24

In order to determine the solubility of the constituents other than santonin in the solvent, a sample was prepared as follows:

Sample BB:

Gluside.....	0.06 gram
Tincture of vanilla.....	1.50 cc.
Cocoa, in fine powder.....	6.00 grams
Sucrose, in fine powder.....	21.00 grams

Materials were passed through an 80-mesh sieve. Samples consisting of 1 gram of material were used.

METHODS.

I.—German Pharmacopeia VI.

This method is open to objections in that it does not state the quantity of solvent to be used.

Weigh out the sample as directed and extract with warm chloroform; filter if necessary through a dry filter. Evaporate the chloroform solution at the temperature of a water bath. Dry at 100°C. to constant weight.

II.—Hagar:

Weigh out the sample, mix with sand or pulverized pumice stone, and extract with chloroform in a Soxhlet or Bailey extractor. Distil off the chloroform and dry the residue at 100°C. to constant weight.

III.—Kropat:

Weigh out the sample, transfer to a 100 cc. glass-stoppered flask, and add 50 grams of chloroform. Shake continuously for 5 minutes and then allow to stand for 12 hours with frequent shaking. Add 20–30 drops of water to the contents of the flask and shake vigorously about 50 times. If necessary, accelerate agglomeration of the mass by the addition of about 0.1 gram of tragacanth and more shaking. Allow to settle for 1–2 minutes and pour off 45 grams of the chloroform-santonin solution into an accurately weighed evaporating dish. After the volatilization of the chloroform on the water bath, place in the drying oven at 100°C. for $\frac{1}{2}$ hour, allow to cool in the desiccator, and weigh.

IV.—Eder-Schneider (Modified):

Triturate the sample with sea sand and transfer the powder to a flask. Shake with 40 cc. of chloroform for 5 minutes. Add 1 cc. of water and shake vigorously for $\frac{1}{2}$ minute. Add chloroform to make to 50 cc., filter through a dry filter, transfer 40 cc. of the filtrate to a tared flask, evaporate the solvent on a water bath, dry the residue for 1 hour at 100°C., cool in a desiccator, and weigh.

Preliminary experiments confirmed the results of Eder and Schneider¹ that santonin could be heated continuously at 100°C. without loss in weight after drying.

COMMENTS BY COLLABORATORS.

L. E. Warren:

Sample B with Method III.—The mixture would not agglomerate sufficiently for pouring, even after the addition of tragacanth. I was obliged to filter through a dry filter and to reject the first 2 cc. of the filtrate.

Sample B with Method IV.—The same conditions met with as in preceding method.

Method IV.—I used sea sand equivalent to one-half the weight of the sample.

¹Schweiz. Apoth. Ztg., 1925, 63: 557.

TABLE 1.
Collaborative results.

COLLABORATOR		SAMPLE A (2.94 %)		SAMPLE AA	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Method I	Kimmel	2.53	2 44	0 14	0 35
	Burlage	2 32	2 28	0 05	0.06+
	Ewing	2.58	0.22
	Warren	2.53	2 41	0.11	0 18
	Average	2.44		0 16—	
Method II	Kimmel	2.27			
	Burlage	2.41	3.10	0 14	0.11
	Ewing	2.53	0 22
	Warren	2.51	2 50	0 32	0 27
	Average	2.55+		0 21+	
Method III	Kimmel	2.74	2.46	..	0 08
	Burlage	3.13	3 19	0 12
	Ewing	2 24	2.42	0 19+
	Warren	2.38	2.40	0.07	0 11
	Average	2.65+		0 11+	
Method IV	Kimmel	2 01	2.44	
	Burlage	2.46	2 47	0 07	0 08—
	Ewing	2.52—	0 20+	
	Warren	2 58	0 07	0 09+
	Average	2.41+		0 10+	

E. O. Ewing:

Of the four methods we are of the opinion that the one under Method II is the best for fat-free mixtures, but really do not consider any of the methods entirely satisfactory.

Method I is not sufficiently explicit in regard to the number of extractions, amount of solvent, or method of extraction. It is essentially the same as Method II, which does give precise directions, and for that reason we think Method I can be immediately eliminated from consideration.

The principal difficulty with Method II, as we see it, is that it will not be applicable to mixtures in which fat is present. Incidentally, in mixtures in which calomel is present, it is advisable to make sure that the chloroform is both dry and neutral.

We have not made an analysis of sample B, but some constituent present apparently throws the determination by method III off very badly. The principal difference in conditions in Methods II and III is the presence of moisture in III. This may possibly have an adverse effect on the solubility of the constituents in the mixture. If we base our opinion upon the results we obtained, the method appears to be open to criticism.

H. M. Burlage:

With Method I four extractions were made, 20, 10, 5, and 5 cc. of solvent being used.

For non-fatty mixtures, agglomeration by methods III and IV was easily accomplished, but with fatty mixtures the emulsions formed would not agglomerate and they were filtered with difficulty.

The comments of the other collaborators cover the experiences met with by the associate referee.

It will be noted that none of the methods was applicable to fatty mixtures. The associate referee regrets that a more favorable report could not be presented and feels that on the basis of this report not one of the methods is sufficiently accurate and practicable to be adopted even as a tentative method.

RECOMMENDATIONS¹.

It is recommended that more time be allowed for further studies of old and new methods for the assay of santonin in fatty and non-fatty mixtures and if possible that such investigation be extended to the assay of santonin-bearing drugs.

REPORT ON ETHER.

By H. M. JOSLIN² (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The past year's work has been concerned in the main with a further investigation of the Somogyi method for the determination of ether in ether-alcohol-water solutions³. In last year's report⁴, a modification of the Somogyi method specifying an alcohol vapor absorption solution surrounded by a constant temperature bath held at 50°C. was suggested. Inasmuch as such a bath is not always readily accessible, efforts were directed towards its elimination. The acid concentration in tube I was changed from 1 + 3 sulfuric acid to 1 + 2 sulfuric acid. In addition, iodometric methods were used to determine the excess dichromate which remained in tube II after the aspiration was complete. The outlets of the vapor-carrying tubes leading into absorption tubes I and II were drawn down so that they presented small openings. In this way very small bubbles issued, insuring efficient washing of the vapors carried through the system. It was found that particular care must be used in the handling and preparation of solutions of ether-alcohol-water and that for this purpose cooled solutions gave the best results.

The modified method follows:

REAGENTS.

Sulfuric acid solution.—Mix 1 volume of concentrated sulfuric acid with 2 volumes of water.

Concentrated sulfuric acid.

Normal potassium dichromate.

0.1 N potassium dichromate.

0.1 N sodium thiosulfate.

Starch indicator.

PROCEDURE.

Set up the apparatus described by Somogyi with the following modifications:

At the beginning of the absorption train insert an ordinary gas washing bottle containing sulfuric acid and having an inlet tube 5 mm. in diameter. Add 150 ml. of sulfuric acid solution to tube I. Mix 50 ml. of normal potassium dichromate with an equal volume of concentrated, cooling the while, and transfer to tube II.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 75.

² Presented by G. C. Spencer.

³ *Z. angew. Chem.*, 1926, 39: 280.

⁴ *This Journal*, 1928, 11: 360

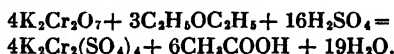
Weigh 0.2–0.4 gram of the cooled distillate to be examined in a glass-stoppered capsule. Introduce the capsule into flask A, close the flask, and start the current of air through the train. So regulate the air stream that 85–95 bubbles of air per minute pass through the gas wash bottle at the beginning of the train. Warm flask A by immersing it in hot water until the glass stopper is expelled. Continue the aspiration of air through the apparatus at the rate specified for 3 hours.

(The contents of tube I may be used for the estimation of alcohol, but no report on this phase of the work will be made at this time.)

Dilute the contents of tube II and make up to a volume of 1000 ml. Take a 25 ml. aliquot and determine the dichromate strength by iodometric methods according to Treadwell and Hall¹.

Calculate the weight of the ether oxidized from the quantity of potassium dichromate decomposed. 1 cc. of 0.1 *N* potassium dichromate = 0.9256 mg. of ether.

The chemical reaction is as follows:



The results obtained by this method are given in the tables.

TABLE 1.

Recovery of anhydrous ether of known weight.

QUANTITY TAKEN	0.1 <i>N</i> DICHROMATE CONSUMED	ETHER RECOVERED	ETHER RECOVERED
mg.	ml.	mg.	per cent
100.9	106.5	98.6	97.7
76.8	82.0	75.9	98.8
129.3	138.3	128.0	99.0
69.6	76.8	71.1	102.1

TABLE 2.

Recovery of ether from water-alcohol-ether solution of known concentration.

QUANTITY TAKEN	ALCOHOL PRESENT	WATER PRESENT	ETHER PRESENT	0.1 <i>N</i> DICHROMATE CONSUMED	ETHER RECOVERED	ETHER RECOVERED
mg.	per cent	per cent	per cent	ml.	mg.	per cent
296.0	42.5	17.35	40.15	126.3	116.9	39.5
257.1	42.5	17.35	40.15	108.4	100.3	39.02
394.2	42.3	25.71	31.97	130.5	120.8	30.64
301.2	42.3	25.71	31.97	98.6	91.3	30.3
230.1	37.24	27.59	35.17	86.5	80.1	34.8
339.0	37.24	27.59	35.17	124.9	115.6	34.1

No report was given on bioassay of drugs owing to the resignation of the associate referee. A paper, entitled "Bioassay of Commercial Pituitary Powers", by McClosky and Munch, was presented. This paper has been published².

No report on fluidextract of ginger was given by the associate referee.

¹ Analytical Chemistry, 6th ed., p. 554.

² *This Journal*, 1929, 12: 135.

REPORT ON EPHEDRA.

By A. E. PAUL and C. K. GLYCART (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referees*.

The extensive physiological and chemical researches recently made by Chen¹ and by Chen and Kao² have shown that the alkaloid ephedrine, isolated from the Asiatic drug plant Mahuang, has remarkable qualities resembling epinephrin, with the added properties of prolonged action, effectiveness when administered by mouth, low toxicity, and stability in solution. Ephedrine is also similar to epinephrin in chemical composition.

Over forty ephedra species distributed throughout the world have been classified; several of these, particularly the American plants, have been found to yield no ephedrine or pseudoephedrine.

Heretofore the knowledge concerning the Chinese botanical sources of ephedrine has been uncertain. However, Read and Liu³ of Peking Union Medical College advise that the material collected and sold as Mahuang consists chiefly of *Ephedra sinica*, Stapf, an entirely new species, and *Ephedra equisetina*. The green stems are the only part of the plant used, since the berries, roots, and woody stalks do not contain ephedrine.

Ephedrine, $C_8H_9 \cdot CHOH \cdot CH(NHCH_3) \cdot CH_3$, occurs as white odorless crystals, soluble in ether, chloroform, alcohol, and water. The water solution is strongly alkaline to litmus. Ephedrine melts at 39°–40°C. The specific rotation, α_D^{20} , in absolute alcohol is levo 6.3°. Pseudoephedrine is the isomer of ephedrine. It is readily soluble in ether and alcohol, but sparingly soluble in cold water. The melting point is 117°–118°C. The specific rotation, α_D^{20} , is dextro 51.2°.

In the past two years results of various ephedra assays ranging from 0.26 to 1.43 per cent have been reported by several investigators, among whom are Masucci and Suto⁴, Schoetzow and Needham⁵, Nielson, McCausland and Spruth⁶, Feng and Read⁷, and Williams⁸.

Certain remarkable chemical properties of ephedrine render the usual methods of assay inapplicable. It has been pointed out by Peterson⁹ that when a water solution of ephedrine hydrochloride or sulfate is shaken with chloroform in the presence of ammonia and the chloroform extract evaporated, ephedrine hydrochloride is obtained. The alkaloid is liberated only when ammonia is present in very large excess, as shown by Feng and Read⁷. Ephedrine being soluble in water, washing the

¹ *J. Am. Pharm. Assoc.*, 1925, 14: 189.

² *Ibid.*, 1926, 15: 625.

³ *Ibid.*, 1928, 17: 339.

⁴ *Ibid.*, 1926, 15: 748.

⁵ *Ibid.*, 1070.

⁶ *Ibid.*, 1927, 16: 293.

⁷ *Ibid.*, 1034.

⁸ *Ibid.*, 1928, 17: 430.

⁹ *Ind. Eng. Chem.*, 1928, 20: 388.

ether extract is inadvisable; the alkaloidal residue cannot be heated without loss due to volatilization even at low temperatures.

The material for the work this year consisted of a 50-pound portion of the crude drug representing an importation of 100 bales from Tientsin, China, invoiced as *Ephedra vulgaris*. A specimen was submitted to G. W. Groff, Bureau of Plant Industry, U. S. Department of Agriculture, Berkeley, Calif., who advised as follows: "I note the product in question was invoiced *Ephedra vulgaris*. This name is synonymous with *Ephedra distachya* L., apparently not known in China. I believe your sample more likely *Ephedra equisetina*, Bunge or *Ephedra sinica*, Stapf. Fruiting material and some notes concerning localities in which collected are extremely desirable for correct identifications".

The following material was submitted to the collaborators: directions for the assay method, a sample of the crude drug in fine powder (60-mesh), an alcohol solution of 0.04 per cent bromthymol blue indicator, and a sealed comparator tube containing bromthymol blue indicator, pH 6.0, for matching the end point in titration.

The directions for the assay are essentially as given in an unpublished method by H. McCausland, with the addition of titration procedure number 2.

EPHEDRA ASSAY.

Place 10 grams of ephedra, in fine powder, into an Erlenmeyer flask. Add exactly 100 cc. of solvent consisting of three volumes of ether and one volume of chloroform, cooled to working temperature after being mixed. Stopper securely and shake. Allow to stand at least 5 minutes, add 5 cc. of 10 per cent ammonia and 0.5 gram of anhydrous sodium carbonate, stopper tightly, and shake the mixture intermittently for 2 hours; then allow to macerate for 4 hours. Decant or filter rapidly a 50 cc. aliquot of the clear supernatant liquid representing 5 grams of the drug, transfer to a separatory funnel, and shake with three portions of diluted sulfuric acid, using 15, 10, 10 cc., etc., until the extraction is complete. Combine the acid solutions in a separatory funnel, neutralize with strong ammonia, then add about 5 grams of anhydrous sodium carbonate, stirring until dissolved. Shake with five portions of ether, using 35, 30, 25, 20, 15 cc., etc., until extraction is complete and combine the ether portions in a second separatory funnel. When clear, decant and filter into a small beaker through a pledget of cotton previously wet with ether.

Titration Procedure No. 1.

Evaporate the solvent to 5 cc. volume on a steam bath with the aid of a fan. Remove from the heat and finish evaporation at room temperature before the fan. Dissolve the alkaloidal residue in 2 cc. neutral alcohol diluted with about 40 cc. of carbon-dioxide-free water. Titrate with 0.02 *N* sulfuric acid to yellow color with bromthymol blue indicator, using standard indicator, pH 6.0.

Titration Procedure No. 2.

Evaporate the solvent to 5 cc. volume on the steam bath with the aid of the fan, and add bromthymol blue indicator and a measured excess of 0.02 *N* sulfuric acid. Cover with a watch glass, return to the steam bath in order to dissolve any alkaloid

adhering to the sides of the beaker, and then evaporate the ether. Titrate the excess acid with 0.02 *N* alkali.

1 cc. of 0.02 *N* acid = 0.0033 gram of ephedrine alkaloids.

The results of the assays and the comments of the collaborators are as follows:

H. McCausland, Abbott Laboratories, North Chicago, Ill.—

TITRATION PROCEDURE

TOTAL ALKALOIDS
per cent

No. 1	0.99
No. 2	1.00

In commenting on the method, I will first say that I consider your reduction in the amount of drug taken, from 15 to 10 grams, to be an improvement, as in the case of samples containing a high percentage of alkaloid an unduly large amount of 0.02 *N* acid is required.

Your directions read "shake intermittently during 2 hours, then allow to macerate for 6 hours". If the assay is started in the morning, it is not possible to proceed with the shaking out until the following morning. Personally, I consider 4 hours' maceration with occasional shaking sufficient, or the test can be started later in the day, shaken at intervals during 2 hours, allowed to stand overnight, and shaken once more in the morning before settling. I was not able, even after long standing, to decant 50 cc. of clear ethereal solution, but found it necessary to filter.

In regard to washing the ether solution with water, I am of your opinion, that it is inadvisable owing to the solubility of the base.

Of the two titration procedures, I prefer No. 1. With No. 2 I noticed, after the final evaporation of the ether, a resinous deposit adhering to the sides of the beaker, which seemed to occlude alkaloid; a second addition of 0.02 *N* acid was necessary to arrive at the yellow shade.

J. B. Williams, Parke, Davis & Co., Detroit, Mich.—

TITRATION PROCEDURE

TOTAL ALKALOIDS
per cent

No. 1	0.957 0.980 0.990
No. 2	1.082 1.063 1.069

The sample No. 2 was assayed by the U. S. P. X method for belladonna, modified by taking 5 grams of drug and using sodium hydroxide solution instead of ammonia. Ether was used for the final extractions, and the volumetric acid was added to the ether solution before evaporation. Results were as follows: 1.195, 1.181, and 1.181 per cent alkaloids. In regard to washing the ether solution, it has been my experience that this is unnecessary provided a good separation is obtained and the ether is passed through an ether-wet pledget of cotton.

H. O. Moraw, Swan-Myers Co., Indianapolis, Ind.—

ALKALOIDS OF EPHEDRA
per cent

0.97
0.95
0.99
1.01

If the aqueous solution is removed from each extraction as completely as possible before transferring, there seems to be no need of washing the combined ether extractions.

It is suggested that at least 10 minutes be allowed after adding the 10 per cent ammonia, before adding the sodium carbonate, to insure complete penetration of the ammonia gas into the ground drug. This procedure was followed on my last two determinations. The fact that they are higher cannot be taken as evidence of greater penetration, but tends to point that way.

No effect of the two different titration procedures could be definitely established on these assays, owing to the possible variation in the quantity brought up to the point of titration. My choice of the two procedures is No. 1 modified as follows: The acid should be added first to prevent possible decomposition of the alkaloid in the presence of alcohol, especially when heated to dissolve from the sides of the vessel. In all titrations which I have made on ephedrine without adding an excess of acid, there has been a further consumption of acid upon adding excess and titrating back. I have noticed an odor of benzaldehyde when the base is treated with alcohol and warmed without excess acid present, indicating possible decomposition.

Leslie Hart, Food, Drug and Insecticide Administration, Chicago, Ill.—

ALKALOIDS OF EPHEDRA		
	A	B
	<i>per cent</i>	<i>per cent</i>
Titration procedure No. 1	(1) 0 80	(1) 0 87
	(2) 0 77	(2) 0 86
	(3) 0 80	(3) 0 90
Average	0 79	0 88
Titration procedure No. 2	0 90	
	0 88	
Average	0 89	

Method 1 A followed directions exactly as stated. Assay 2 of this group of results was evaporated just to the water stage; that is, the ether was completely volatilized.

Method 1 B was a continuation of A, in which excess acid was added, and the solution was warmed on the steam bath 20 minutes and titrated back with 0.02 *N* alkali to pH 6.0, a comparator tube being used to match the end point. There is evidently further consumption of acid by the back titration method. It will be noticed that this modification checks Method 2, as outlined by the referee.

It was noticed that about 1 cc. of 0.02 *N* acid was required to change the color of the indicator from pale green to the yellow specified in the directions. This is equivalent to about 0.07 per cent difference.

F. C. Synkovich, Food, Drug and Insecticide Administration, Chicago, Ill.—

EPHEDRA ALKALOIDS BY TITRATION PROCEDURE NO. 1	
	<i>per cent</i>
	1 069
	0.96

The method was followed with one exception: the ephedra was allowed to macerate overnight instead of 6 hours as directed.

G. L. Jenkins, University of Maryland, Baltimore, Md.—

EPHEDRA ALKALOIDS	
TITRATION PROCEDURE NO. 1	TITRATION PROCEDURE NO. 2
<i>per cent</i>	<i>per cent</i>
1.24	1.45
1.36	1 33

Procedure No. 2 is not practical, for all ammonia present in the residue is neutralized by the sulfuric acid and is, therefore, calculated as alkaloid. The tenacity with which alkaloids retain ammonia has been overlooked to such an extent in these assay procedures that they do not appear to be accurate.

DISCUSSION.

The reports of the collaborators show that the method is readily workable and that the results are within acceptable limits of accuracy, considering the marked chemical properties of the alkaloid ephedrine.

Williams obtained a higher percentage of alkaloids by using sodium hydroxide instead of ammonia and sodium carbonate, as described in the method given.

Moraw advises that at least 10 minutes be allowed after adding 10 per cent ammonia to insure complete penetration of the ammonia gas in the ground drug. It appears that the effect of these two modifications should be studied.

RECOMMENDATIONS¹.

It is recommended—

(1) That the ephedra assay be further studied and that certain modifications be included, as suggested in the comments of the collaborators.

(2) That qualitative and quantitative methods for the determination of ephedrine and pseudoephedrine in pharmaceuticals be studied next year.

REPORT ON PILOCARPINE IN TABLETS.

By T. F. PAPPE (U. S. Food, Drug and Insecticide Administration, Baltimore, Md.), *Associate Referee*.

The literature on pilocarpine is meager, and, so far as the associate referee could find, gives no method for its accurate determination. The alkaloid, as well as some of its salts, is very hygroscopic. It is also volatile and rather easily decomposed. Pilocarpine is very soluble in water as well as in the usual alkaloidal solvents.

The literature states that fixed alkali converts pilocarpine into an acid, and that from such a solution other alkaloids of a non-phenolic character may be removed. The proper conditions for its determination in this way were not obtained, as the alkaloid was slowly removed from the alkaline solution by chloroform. However, inasmuch as there appeared to be no evidence of pilocarpine being dispensed in conjunction with other alkaloids, an adaptation of the usual alkaloid extraction method was tried out.

The details of the method are as follows:

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1920, 12: 76.

After determining the average weight per tablet, grind a sufficient number of the tablets, mix thoroughly, and weigh out a portion approximately equivalent to 1 grain of the salt. Dissolve in 10 cc. of water, add 1 cc. of dilute ammonium hydroxide (1 + 9), and shake out rapidly with 20 cc. of chloroform. Repeat the extraction, using 15 cc. of chloroform, and then complete the extraction with successive 10 cc. portions. Filter each portion of the chloroform as drawn off through a pledget of cotton and combine in a 250 cc. beaker, finally washing the stem of separator and funnel with chloroform. Evaporate on the steam bath until the chloroformic solution measures about 5 cc. Add 20 cc. of 0.02 *N* sulfuric acid and evaporate the remainder of the chloroform. Titrate any excess acid with 0.02 *N* of sodium hydroxide, using 1 drop of methyl red indicator. The end point is not particularly sharp, but it can be arrived at with care.

1 cc. of 0.02 *N* H_2SO_4 = 0.004893 gram of $\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2 \cdot \text{HCl}$.

This method, together with a suggestion of the alternative use of bromphenol blue as an indicator was sent to ten collaborators. Merck's pilocarpine hydrochloride was used in the preparation of a tablet mass in the proportion of 1 to 3 milk sugar. The pilocarpine hydrochloride assayed 97.31 per cent with methyl red indicator, and contained 2.77 per cent moisture. The tablet mass accordingly contained 24.32 per cent of pilocarpine hydrochloride. Portions of this mass were forwarded to the ten collaborators, and the following reports were received:

Pilocarpine in tablets.

ANALYST	METHYL RED INDICATOR		BROMPHENOL BLUE INDICATOR	
	<i>per cent</i>	<i>average</i>	<i>per cent</i>	<i>average</i>
J. A. Riley	24.17}	24 06	24 23}	24.27
New York Station	23 95}		24 31}	
E. H. Grant	24 55}	24 71	25 44}	25 49
Baltimore Station	24.87}		25.53}	
E. O. Eaton		26 00		
San Francisco Station				
A. H. Fiske	23.98}	24.11		
Eli Lilly & Co.	24.23}			
Indianapolis, Ind.				
D. M. Copley			25 26}	25.18
Norwich Pharmacal Co.			25.10}	
Norwich, N. Y.				
L. E. Warren			25 23}	25.22
Drug Research Unit			25 23}	
Washington, D. C.			25.20)*	
A. W. Hanson	24.95}	24 87	24.79}	24.96
Minneapolis Station	24.79}		25.12}	
F. C. Synkovich			25.7 }	25.60
Chicago Station			25 2 }	
			25.9 }	
H. R. Watkins	26.01}	26.09	26.01}	25.93
Drug Control Laboratory	26.17}		25.85}	
Washington, D. C.				
T. F. Pappe	24.29}	24.31	25.78}	25.73
	24.32}		25.67}	
Maximum		26 09		25 93
Minimum		24 11		24 27
Average		24.88		25.30

* Used continuous extractor.

Riley and Watkins expressed a preference for bromphenol blue indicator, and Grant and the associate referee preferred methyl red. Hanson also suggested evaporation of the chloroformic solution almost to dryness, which may cause loss of pilocarpine and is not necessary, as it was repeatedly demonstrated that all ammonia is removed under the procedure outlined.

It will be seen that the results with methyl red more nearly approximate the theoretical content, only one operator obtaining a satisfactory result with bromphenol blue. In fact, if the high results with methyl red, 26.00 per cent and 26.09 per cent, are disregarded, the average of the remaining five reports is 24.41 per cent, which agrees with the theoretical amount.

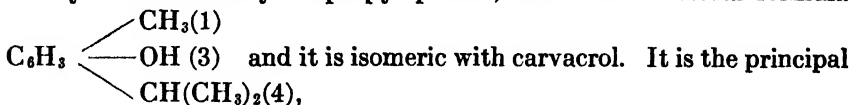
The work indicates that satisfactory determinations of pilocarpine in simple tablet masses can be made by the method outlined when methyl red indicator is used. It is recommended that this method be adopted as tentative¹.

REPORT ON THYMOL.

By LESLIE HART (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

The U. S. Pharmacopeia does not specify any assay for thymol, nor has this association recognized any method for the quantitative estimation of this product. It was therefore recommended by the Referee on Drugs that this study be undertaken.

Thymol is a methyl isopropyl phenol, with the structural formula



constituent of oil of thyme and is also found in the essential oils of ajowan, horse-mint, wild thyme, certain varieties of origanum, etc. It is prepared by extraction from the essential oils by dilute caustic soda. The aqueous layer is separated and treated with dilute acid, and the thymol rises to the top as an oily layer. It may also be prepared synthetically from *p*-cymene or piperitone.

Previous to submitting a new method for study, an investigation was made by the associate referee of the accuracy of the various methods found in the literature. The methods studied included the iodine absorption method of Messinger and Vortmann², the bromine absorption method of Koppeschaar³, and various modifications of these basic reactions⁴, which are applicable to all phenolic compounds.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 76.

² *Ber.*, 1890, 23: 2753.

³ *Z. anal. Chem.*, 1876, 15: 233.

⁴ *J. Ind. Eng. Chem.*, 1918, 5: 389, 831; *Pharm. Rev.*, 19, 14: 221; *Z. anal. Chem.*, 1902, 41: 227.

In the case of the iodine method, it was found that comparatively slight variations in the quantity of alkali present and of the quantity of iodine used in excess of that required by theory (4 atoms of iodine to 1 molecule of thymol) gave wide differences in the percentage of thymol. In the case of the Koppeschaar bromine method, it was also found that the quantity of bromine solution present in excess of that required for the formation of dibromthymol and the time allowed before titrating the excess bromine materially affected the results. This confirmed a report by Seidell¹, who endeavored to apply these methods to salicylic acid and later to thymol.

As a result of the study of the bromination reaction it was thought that thymol could be determined by direct titration with Koppeschaar's solution, in the presence of strong acid, the end point being observed by the bleaching of the red color produced by methyl orange in acid solution when acted upon by bromine in excess of that required for the formation of dibromthymol. This was suggested by Seidell's method for the determination of salicylates, in which salicylic acid is titrated directly with 0.2 *N* bromide-bromate solution in the presence of warm, strong hydrochloric acid solution. His end point was the appearance of a yellow color due to excess bromine, permanent for 5 minutes. The use of the bleaching effect of bromine on methyl orange increased the sensitivity of the reaction and also decreased the time required for the assay. In this way it is also possible to control the amount of excess bromine present if it is desired to make a back titration with thiosulfate after the addition of potassium iodide. As stated above, it was found that differences in the quantity of bromine present in excess of that required by the thymol gave a material difference in the results obtained. In this manner the amount of excess could be definitely controlled.

A sample of thymol, labeled U. S. P., prepared by a reputable manufacturer and bought on the open market, was tested and found to conform to the non-volatile residue requirements of the U. S. Pharmacopeia. It was powdered and sent to the various collaborators, together with the two methods described in this report. On the whole, excellent results were obtained by the collaborators, as is shown by the table.

Two volumetric methods were submitted to the collaborators for study. Both methods were based on the reaction that occurs when nascent bromine, furnished by a standard bromide-bromate solution, reacts with thymol, in the presence of hot, strong hydrochloric acid, according to the equation— $\text{C}_6\text{H}_3\text{CH}_3 \cdot \text{CH}(\text{CH}_3)_2 \text{OH} + 4\text{Br} = \text{C}_6\text{HBr}_2 \cdot \text{CH}_3 \text{CH}(\text{CH}_3)_2 \text{OH} + 2 \text{HBr}$. The methods differ only in the recognition of the completion of the bromination.

The end point of Method A is observed by the destruction of the red color of methyl orange indicator by slight excess of bromine. Method B

¹*J. Am. Chem. Soc.*, 1909, 31: 1168; *Am. Chem. J.*, 1912, 47: 508

is a back titration using the familiar starch-iodine end point. These methods have been published¹. The results are shown in the table.

TABLE 1.
Collaborative results.

COLLABORATOR	METHOD "A"				METHOD "B"			
	No. of Assays	Maximum	Minimum	Average	No. of Assays	Maximum	Minimum	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
M. R. Thompson . . .	2	98.06	97.69	97.88	2	98.06	97.87	97.96
L. N. Markwood	4	99.79	99.59	99.71	4	99.79	99.39	99.59
W. F. Kunke	5	100.5	99.7	100.01	5	100.3	99.0	99.66
R. S. Roe	5	99.05	99.80	99.21	3	101.49	99.05	100.00
Jos. Calloway, Jr.	2	98.7	98.3	98.5	2	97.5	97.5	97.5
Quick Landis	2	99.71	99.30	99.51	2	100.42	99.94	100.18
Howard A. Jones	5	99.2	100.0	99.6	5	98.9	99.6	99.4
F. C. Synkovich	2	100.13	99.79	99.96	2	100.02	99.64	99.83
Glen L. Jenkins	4	99.04	99.83	99.34	5	100.02	99.27	99.69
University of Maryland								
Student No. 1 . . .	2	100.45	100.44	100.45	2	100.41	100.21	100.31
Student No. 2	3	98.35	96.93	97.65	3	98.93	97.77	98.29
Student No. 3	6	93.70	98.23	96.80	6	100.98	95.06	97.52
L. S. Crosby	5	98.74	98.14	98.38	5	96.68	98.22	97.38
Crosby, using methyl red indicator	2	99.75	99.34	99.55				
Crosby: Report No. 2	2	99.63	99.25	99.44	2	98.47	98.28	98.38
Crosby, using methyl red indicator	2	101.20	100.49	100.85				
Leslie Hart:								
Report No. 1	6	100.5	99.7	100.15	5	101.0	98.6	100.07
Report No. 2	10	100.43	99.14	99.87	10	100.15	98.38	99.01
Report No. 3	3	99.70	98.72	99.21	3	99.84	99.60	99.76

REMARKS:

Kunke: Two assays, run one week later with aliquots from same solution, gave an average of 99.9 per cent for Method A and 99.6 per cent for Method B.
Two assays, using 0.25 gram sample and 0.2 *N* bromine solution gave 100.27 per cent for Method A and 100.05 per cent for Method B.

Crosby: Report No. 2 was made after taking precautions to swirl constantly during first titration.

Hart: Report No. 1: Used 0.05 gram sample, titrated with 0.1 *N* bromine.
Report No. 2: Used 0.10 gram sample, titrated with 0.1 *N* bromine.
Report No. 3: Used 0.25 gram sample, titrated with 0.2 *N* bromine.

¹ *This Journal*, 1929, 12: 54.

COMMENTS BY COLLABORATORS.

L. S. Crosby.—The solution should be constantly swirled during the first addition of the bromine solution. The method as submitted did not call for swirling until after the addition of the indicator, whereas it appears to be equally, if not more important when the greater part of the bromine is being added before the addition of the indicator.

W. F. Kunke.—The methods are satisfactory, rapid, and convenient. Method A is preferred, being quicker.

Glen L. Jenkins.—Results obtained by students show the variation obtained by comparatively inexperienced analysts. The procedure in Method A does not give a sharp end point, especially if the contents of the flask become slightly cool. Method B appears to be better adapted for general work in that the end point is more definite and should yield more concordant results in the hands of various workers.

R. S. Roe.—Method A gave more consistent results, and takes less time, after a few preliminary titrations, for the analyst to become accustomed to the end point.

Howard A. Jones.—Method A.—The end point is rather poor. It is necessary for an analyst who has never used this method to repeat the determination a number of times before consistent results are obtained. After some experience, however, it seems to give good results. Method B is free from this objection, since the end point is distinct, and is one familiar to most analysts. It is probable that a satisfactory method might be worked out eliminating the titration in method A and proceeding directly to method B. It is suggested that a small quantity of chloroform be added toward the end of the thiosulfate titration. 0.1 cc. of 0.1 *N* bromine solution is equivalent to 0.4 per cent thymol (when determining pure thymol). Accuracy would be increased by using more dilute bromine, or by increasing the amount of sample.

Quick Landis.—Either method is satisfactory to me personally, although B might lend itself to a large number of simultaneous results more readily.

Joseph Calloway, Jr.—The details are apparently carefully worked out and I have no comments to offer. However, the method is not specific to thymol. Carvacrol, if present, will be calculated as thymol.

L. N. Markwood.—Method A is satisfactory. With a little practice there is no difficulty getting an end point. Method B is also satisfactory. I am inclined to prefer A, as it requires only one standard solution.

M. R. Thompson.—The methods gave very concordant results. I suggest adding 1 cc. of chloroform before the end point is reached in the thiosulfate titration of Method B.

DISCUSSION OF RESULTS.

The average results obtained by the two methods are quite concordant. Nine collaborators on Method A and eight on Method B obtained results well over 99 per cent, or closer than 1 per cent of the theoretical.

It was realized by the associate referee that with the quantity of sample taken for titration, one drop of the titrating solution would represent a rather large quantity, proportionally, of thymol. It was decided to use 0.1 *N* solution, because this is the strength commonly used in pharmaceutical laboratories. It was desired to keep the volume of titrating solution down to about 30 cc. in order to avoid excessive cooling of the mixture, thus slowing up the end point. However, the associate referee obtained results by using 0.05 gram and a 0.1 *N* bro-

mine solution, and also by using 0.25 gram and 0.2 *N* bromine that agree well with the results obtained when the methods submitted to the collaborators were used.

If Method B is used independently of Method A, the preliminary titration in hot solution with 0.1 *N* bromine and methyl orange as an indicator should be made, so as to regulate the quantity of excess bromine present for the back titration. Also, the solution should be titrated with standard thiosulfate immediately after the addition of the bromine solution. As mentioned previously, the quantity of excess bromine and time of standing materially influence the accuracy of the results.

The assay of thymol by either of the two methods requires very little time. If the titrating solutions are standardized, duplicate determinations with both Methods A and B may be completed in a half hour's time.

While the two methods gave concordant results, the associate referee believes that Method A is inherently the better method, as it eliminates the effect of excess bromine and the consequent time factor.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the direct titration method, designated as "A" in this report, be adopted as a tentative method.
- (2) That further study be made on the determination of thymol in the presence of other substances.

REPORT ON MENTHOL.

By F. L. ELLIOTT (U. S. Food, Drug and Insecticide Administration, Baltimore, Md.), *Associate Referee*.

No methods for the analysis of menthol have been considered by the association, and a review of literature fails to disclose any satisfactory method. The usual qualitative test for small quantities is its characteristic odor. The physical constants are described by several writers.

The United States Pharmacopeia contains a method for the analysis of menthol in oil of peppermint, and this method slightly modified, together with a sample of U. S. P. menthol, was submitted to several collaborators.

METHOD.

Weigh 5 grams of menthol in an acetylation flask of 100 cc. capacity, and add 10 cc. of acetic anhydride and 1 gram of powdered anhydrous sodium acetate. Boil the mixture gently for 1 hour, cool, and disconnect the flask from the condenser, transferring the mixture to a small separatory funnel. Rinse the acetylation flask with three

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 76.

successive 5 cc. portions of warm distilled water and add the rinsings to the separatory funnel. When the liquids have completely separated, remove the aqueous layer, and wash the remaining oil with successive portions of sodium carbonate test solution, diluted with an equal volume of distilled water, until the mixture is alkaline to 2 drops of phenolphthalein test solution. Dry the resulting oil with fused calcium chloride and filter. Transfer 4-5 cc. of the dry acetylated oil to a tared 100 cc. Erlenmeyer flask, note the exact weight, add 50 cc. of 0.5 *N* alcoholic potassium hydroxide, connect the flask with a reflux condenser, and boil the mixture on a water bath for 1 hour. Allow the mixture to cool, disconnect the flask from the condenser, and titrate the excess of alkali with 0.5 *N* sulfuric acid, using 10 drops of phenolphthalein test solution as indicator. Calculate the percentage of menthol by the following formula:

$$\text{Percentage of total menthol} = \frac{A \times 7.811}{B - (A \times 0.021)}.$$

A is the result obtained by subtracting the number of cc. of 0.5 *N* sulfuric acid required in the above titration from the number of cc. of 0.5 *N* alcoholic potassium hydroxide originally taken; *B* is the weight of acetylated oil taken.

Sodium carbonate test solution.—Dissolve 12.5 grams of monohydrated sodium carbonate in sufficient distilled water to make 100 cc.

The results submitted are as follows:

	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
E. C. Merrill United Drug Co., Boston, Mass.	100 52	100 53	100 48
Charles F. Bickford U. S. Food, Drug and Insect. Adm. New York, N. Y.	100.2	100.7	
Leslie Hart U. S. Food, Drug and Insect. Adm. Chicago, Ill.	99 94	100 12	100 4
Hugh H. Mottern U. S. Food, Drug and Insect. Adm. Baltimore, Md.	(a) 101.2 (b) 98 52	(a) 100 5	(a) 101 8
Howard R. Watkins U. S. Food, Drug and Insect. Adm. Washington, D. C.	98 52	98 65	
Fred L. Elliott U. S. Food, Drug and Insect. Adm. Baltimore, Md.	99.8	100 2	100 3

COMMENTS OF COLLABORATORS.

E. C. Merrill.—Figures obtained are somewhat high. Possibly the factors are at fault, or perhaps same could be corrected by running this method against menthol of known purity.

Leslie Hart.—The acetylated residue from one assay was allowed to stand over the week-end in contact with anhydrous calcium chloride. This procedure resulted in a pasty mass, which was extracted with ether, the ether evaporated, and saponification value determined on the residue. The menthol content of this assay was 99.98 per cent.

The details of the method have been well worked out, and no difficulty was found with the procedure. It required quite a bit of washing to extract the acetic anhydride. I would suggest that 10 cc. of water be added to the contents of the flask after acetylating, the mixture boiled 10 or 15 minutes to break up the acetic anhydride and then transferred to a separatory funnel, the aqueous layer separated, and the oily layer

washed once with distilled water. One or two washings with sodium carbonate solution will then suffice to remove the acetic acid remaining in the oils.

Hugh H. Mottern.—Results marked (a) were obtained by average washing of acety-lized oil, (b) by further washing.

CONCLUSIONS.

The results of collaborators are fairly satisfactory. The variations are apparently due to the difficulty of removing *all* the acetic anhydride from the acety-lized oil. It is recommended that further study be made of the difficulties in removing acetic anhydride from the acety-lized oil¹.

REPORT ON BROMIDES-CHLORIDES.

By H. WALES (Drug Control Laboratory, Food, Drug and Insecticide Administration, Washington, D. C.), *Associare Referee*.

Inasmuch as this is a new topic, only a preliminary investigation was made this year. From a study of methods described in the literature, the most promising appears to be that of Winkler².

In brief this method is as follows:

(a) Strongly acidify the solution containing the bromide and chloride with sulfuric acid and place in a flask connected with a condenser. Immerse the delivery end of the condenser in a solution of sulfurous acid. Heat the bromide solution to boiling and add permanganate solution slowly until the pink color persists for 1 minute. After all the bromine has been distilled off, boil the sulfurous-hydrobromic acid solution until free from sulfur dioxide. Strongly acidify this solution with sulfuric acid and titrate the bromide at the boiling point with standardized permanganate.

(b) If the bromide solution is free from reducing substances and contains less than 25 per cent of chloride, titrate directly with permanganate without the preliminary separation.

Winkler states that the bromine may be collected in hydrogen peroxide instead of sulfurous acid and the resulting hydrobromic acid titrated with alkali or silver nitrate, or determined gravimetrically. The bromine may also be collected in dilute sodium hydroxide and the resulting hypobromite determined by any of the usual methods.

If ammonium salts are present, they must be removed by boiling with alkali before titration. Iodides are removed by boiling the acidified solution with ferric sulfate or chloride.

The present study was confined to the determination of bromides in the presence of chlorides only, the modifications necessary when iodides or ammonium salts are present being left for future study. Sulfurous acid was used for the collection of the bromine distilled off during the reaction.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 76.

² *Z. angew. Chem.*, 1915, 28: 1, 477.

The following results were obtained:

USED		0.1 N KMnO ₄ REQUIRED	K Br FOUND	
K Br	NaCl			
gram	gram	cc.	gram	per cent
<i>Direct titration.</i>				
0.3011	25.41	0.3024	100.43
0.3011	25.22	0.3002	99.70
0.2409	20.23	0.2408	99.95
0.2409	0.0591	20.26	0.2411	100.08
0.2409	0.1182	20.30	0.2416	100.29
<i>Titration after distillation of bromine.</i>				
0.2409	1.0	20.13	0.2396	99.46
0.2409	1.0	20.41	0.2429	100.83

The associate referee believes that this method is worthy of further consideration and therefore recommends that the study be continued for the coming year¹.

REPORT ON CHENOPODIUM OIL.

By E. K. NELSON (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The present assay method for the determination of ascaridole in chenopodium oil prescribed in U. S. Pharmacopeia X, which was devised by the associate referee, depends on the solubility of ascaridole in 60 per cent acetic acid, the terpenes remaining undissolved.

This method has been criticised by Humphrey Paget² as being inapplicable to oils adulterated with oils containing oxygenated constituents which may also be soluble in 60 per cent acetic acid, for example, eucalyptus oil. In other words, according to Paget the present U. S. P. method, while useful in the assay of authentic oils, may give misleading results with adulterated oils.

In view of Paget's observations, the associate referee conducted experiments with the titanium trichloride method proposed by him, using his factor for ascaridole.

The determination was effected by heating 10 cc. of a 1 per cent solution of the oil under examination with 50 cc. of a standardized solution of titanium trichloride. One gram of chenopodium oil was diluted with 96 per cent alcohol to 100 cc., and to 10 cc. of this in a flask through which a current of carbon dioxide was passing, an excess of titanous chloride, about 50 cc., was added; the flask was then closed with a Bunsen valve, and its contents were heated almost to boiling for 1-2

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 76.

² *Analyst*, 1926, 51: 170.

minutes. If the pale, violet color of the titanous chloride disappeared, more of the reagent was added to insure the presence of an excess. About 1 cc. of 5 per cent potassium thiocyanate was then added, and the solution was titrated back with a standard solution of iron alum until a permanent faint red color was obtained. The quantity of iron used, calculated in terms of titanous chloride, gave by difference the quantity of titanous chloride oxidized. By experiment Paget found that 0.1 gram of pure ascaridole oxidized 0.1277 gram of titanous chloride.

The results obtained by this method on samples of ascaridole, chenopodium oil, and a mixture of chenopodium oil, turpentine, and eucalyptus oil are as follows:

(1) Sample 5276, ascaridole from Klee Bros., which is entirely soluble in 60 per cent acetic acid, oxidized titanous chloride to the extent of 0.1266 gram per 0.1 gram of sample; $\frac{0.1266 \times 100}{0.1277} = 99.1$ per cent ascaridole.

(2) A sample of chenopodium oil from a New York dealer returned 85.0 per cent of ascaridole by the U. S. P. method. A 0.1 gram sample oxidized 0.1040 gram titanium trichloride; $\frac{0.1040 \times 100}{0.1277} = 81.4$ per cent ascaridole.

(3) A sample of chenopodium oil from a distiller in Maryland afforded 69 per cent of ascaridole by the U. S. P. method, while 0.1 gram oxidized 0.08193 gram of titanium trichloride; $\frac{0.08193 \times 100}{0.1277} = 64.1$ per cent.

(4) A mixture of 50 per cent of chenopodium oil from a New York dealer, showing 85 per cent ascaridole by U. S. P. assay, with 20 per cent of turpentine and 30 per cent of eucalyptus oil gave 67 per cent ascaridole by the U. S. P. method; 0.1 gram oxidized 0.05517 gram of titanium trichloride; $\frac{0.05517 \times 100}{0.1277} = 43.2$ per cent ascaridole.

The lower results obtained on genuine oils by the Paget method are attributable to the fact that ascaridole glycol and other transformation products of ascaridole that are soluble in 60 per cent acetic acid do not react with titanous chloride.

The desirability of the oxidation method in the examination of oils for adulteration is strikingly shown by the last experiment. The U. S. P. assay method would have passed this oil as meeting the requirement for ascaridole content, whereas the oxidation method plainly shows inferiority.

Further study of the Paget method, with a view to its adoption as an official method and its insertion in the next revision of the U. S. Pharmacopeia, is recommended¹.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 76.

REPORT ON SABADILLA.

By F. C. SYNKOVICH (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.), *Associate Referee*.

In a Service Regulatory Announcement, January 26, 1916, the Bureau of Chemistry issued a tentative standard as a guide for the officials of the Department of Agriculture in the enforcement of the Food and Drugs Act, that sabadilla seed (*Sabadilla officinalis*) contain not less than 3.0 per cent of alkaloids. No assay was given.

Five alkaloids—cevadine, veratridine, sabadilline, sabadine, and sabadinine—are reported to be present in sabadilla seeds¹. Of these alkaloids, cevadine and veratridine are of more importance therapeutically. Certain authorities are not in agreement with regard to the quantity of alkaloids in the seeds. The U. S. Dispensatory, 21st ed., states that cevadilla contains about 0.3 per cent of alkaloids. The National Standard Dispensatory, 3rd ed., states that the important constituent of sabadilla is about 1 per cent of several alkaloids. Wright and Luff² obtained an equivalent of 0.6–0.7 per cent of basic derivatives. Katz³ found between 2.24 per cent and 5.12 per cent of alkaloids in ten samples of seeds. Caesar and Loretz⁴ obtained 3.81–5.20 per cent and Fromme⁴ found the alkaloidal content of different samples of seeds to range from 4.11 to 5.37 per cent.

SAMPLE.

The material used for the preliminary work and that sent to the collaborators was part of an import shipment offered for entry through the Chicago Station. Examination showed that in size and shape the seeds answered the description for sabadilla given in the Dispensatory. They were practically all sound, and the ash content was found to be 2.8 per cent. The sample was ground, and all material was passed through a 20-mesh sieve. A finer mesh was not taken owing to the presence of fatty matter, which made further grinding difficult.

METHOD.

The proposed method is essentially the U. S. P. X Type Process A for alkaloidal assays. The alkaloids are determined gravimetrically.

REAGENTS.

- (a) *U. S. P. ether*.
- (b) *Ammonia*.—10 per cent solution.
- (c) *Tartaric acid*.—3 grams to 100 cc.
- (d) *Sodium carbonate*.—Saturated aqueous solution.

¹ National Standard Dispensatory, 3rd ed., p. 1745.

² *J. Chem. Soc.*, 1878, 33: 338.

³ Hager's *Handbuch der Pharmazeutischen Praxis*. Ergänzungsband, 1908, p. 38.

⁴ *Jahresber. Pharm.*, 1913, 48: 38.

PROCEDURE.

Weigh 10 grams of the sample, coarsely powdered, into an Erlenmeyer flask, preferably fitted with a ground-glass stopper. Add 100 cc. of ether, accurately measured, stopper securely, and shake.

Let stand for 5 minutes, add 10 cc. of 10 per cent ammonia, shake intermittently during 2 hours, and allow to stand overnight. Shake again several times and allow the suspended matter to settle.

Decant an aliquot into a 50 cc. volumetric flask. Filter the solution through cotton into a separatory funnel, wash flask and cotton with ether, and add rinsings to the separator.

Extract with the tartaric acid solution, using 20, 15, 15, 10, and 5 cc. portions, respectively. Test for complete extraction with Mayer's reagent. Combine the tartaric acid solutions in another separatory funnel. Wash the combined acid solutions with 15 cc. of ether, wash the ether with 10 cc. of water, and add the water to the acid solution.

Neutralize with the sodium carbonate solution, add 3 cc. in excess, and extract the alkaloids with 30, 25, 25, 20, and 10 cc. portions of ether. Combine the ether extracts and wash with 5 cc. of water, washing the water in turn with 15 cc. of ether. Add the ether to the other extractions, allow to settle, and draw off the remaining water into the alkaline solution. Dry out the stem of the separatory funnel and run the ether into another separatory funnel having a stem filled with cotton previously saturated with ether. Filter into a weighed beaker, wash both separatory funnels, and allow the ether washings to drain through the cotton. Extract the alkaline solution with an additional 20 cc. of ether, filter through cotton into a separate beaker, and evaporate. If a residue is obtained, dissolve it in ether and add to the main bulk of alkaloids. (A negligible residue at this point denotes complete extraction.) Evaporate the combined ether extracts on a steam bath, treat the residue with several cc. of alcohol, evaporate, and dry the residue to constant weight at 100°C. The final weight represents the alkaloids in 5 grams of seeds.

EXPERIMENTAL TITRATION OF THE ALKALOIDS.

The alkaloids extracted by the method given were tested for fixed alkali by the associate referee. The dry residue was treated with 5 cc. of water. The water was poured off and titrated with 0.02 *N* sulfuric acid, methyl red being used as the indicator. There was a titration of about 0.1 cc. acid, but this was probably due to a small amount of alkaloids dissolved by the water, for a test with Mayer's reagent was positive. The water-insoluble residue was dissolved in alcohol and titrated with 0.02 *N* sulfuric acid, methyl red being used as the indicator. The factor used, 1 cc. of 0.02 *N* acid = 12.5 mg. of alkaloids, was found in arzneidrogen by H. Zörnig¹.

COMMENTS.

Earl L. Anderson.—Results 1, 2, and 3 were obtained by following the method, care being taken to prevent loss of ether by evaporation. With results 4 and 5 no particular attempt was made to prevent loss by evaporation of ether. After ether and ammonia had been added, the flask and contents were weighed and again made to weight with ether just before taking the aliquot. It was found that there was considerable loss of

¹ *Pharm. Acta Helv.*, 1926, 1: 34.

Results obtained by the associate referee.

	<i>per cent</i>
By the proposed method.....	5.04
	4.96
	5.12
The alkaloids titrated with standard acid.....	4.92
	4.85
	5.04

Results by collaborators.

Earl Anderson, Baltimore, Md.....	(1) 5.30
	(2) 5.06
	(3) 5.19
	(4) 5.24
	(5) 5.17
Leslie Hart, Chicago, Ill.	(1) 4.99
	(2) 5.13
	(3) 5.08
Wm. F. Kunke, Chicago, Ill.....	(1) 5.03
	(2) 4.93
	(3) 5.00
Quick Landis, San Francisco, Calif.....	(1) 5.30
	(2) 5.34
L. N. Markwood, New York, N. Y.....	(1) 5.02
	(2) 5.04
Robert S. Roe, Chicago, Ill.....	(1) 5.02
	(2) 5.16
Frank A. Spurr, Washington, D. C.....	(1) 5.078
	(2) 4.96
	(3) 5.16

ether, although glass-stoppered Erlenmeyer flasks were used. The ether extractions from the sodium carbonate solution were washed with water, which, in turn, was washed with ether and the ether was added to the other extractions (4 and 5).

Wm. F. Kunke.—The method was modified with the associate referee's permission, by using a mixture of two parts of chloroform and one part of ether in place of ether, as the solvent for extracting the alkaloids from the alkaline tartrate mixture. This solution was used for convenience. Also the combined chloroform-ether extraction was washed with 1 cc. of water. Some emulsion was formed, although vigorous shaking was avoided.

Quick Landis.—Ether was allowed to stand on the seeds for about 48 hours. The third ether extract of the neutralized extract gave no residue upon evaporation.

L. N. Markwood.—The method is satisfactory and I have no special comment to offer. I will say, however, that I was surprised to find it took four periods of 30 minutes each to bring the alkaloids to constant weight at 100°C.

Robert S. Roe.—The last extraction "lagged" considerably. I am wondering whether chloroform or a mixture of chloroform and ether could not be substituted satisfactorily for the ether used in the last extraction. It would materially lessen the work involved in drawing off the extractions.

Frank A. Spurr.—Four determinations were made. The "alkaloids" appeared to contain substances other than the alkaloids. The results reported are based on the ether-soluble portion. The ether-insoluble portion was examined, and sodium carbonate was identified.

DISCUSSION.

Loss of ether during maceration was reported by one of the collaborators. Upon allowing the mixture to stand overnight, as directed in the method, the associate referee found losses in weight varying from 10 to 20 mg. in using three different glass-stoppered Erlenmeyer flasks.

A precautionary measure of washing the ether extraction with water was added to the method.

The alkaloids obtained by the method when the alcohol is evaporated have a transparent amorphous appearance. On drying they further harden to a varnish-like mass. They give the color tests for veratrine described in the U. S. Dispensatory.

The gravimetric estimation was chosen because there are five alkaloids in the seeds, and an average factor for the value of the acid volumetric solution may not express the true total of the alkaloids if they are present in varying proportions in different samples. As a basis of comparison, however, the associate referee titrated several of the alkaloidal residues. It was apparent from the results obtained that they did not contain substances other than alkaloids.

The results obtained by the collaborators show that the method yields consistent results.

It is recommended that the method be adopted as tentative¹.

¹ For report of Subcommittee B and action of the association, see *This Journal*, 1929, 12: 77.

CONTRIBUTED PAPERS.

THE SOLUBILITY OF SODIUM AND POTASSIUM HYDROXIDES IN METHYL AND ETHYL ALCOHOLS.

By A. G. MURRAY (Food, Drug and Insecticide Administration,
Washington, D. C.).

At the last meeting of the Association of Official Agricultural Chemists, in connection with a paper on the determination of chloroform and carbontetrachloride there arose the question of the solubility of sodium and potassium hydroxides in methyl and ethyl alcohols. The available solubility tables and other reference works consulted contain very little information on this subject; accordingly experiments for the purpose of determining these solubilities were inaugurated. Since the data were desired for practical purposes only it was not considered necessary to observe such precautions as would have been observed if a high degree of accuracy had been desired.

Sodium and potassium hydroxides in excess were placed in bottles of ethyl and methyl alcohols and allowed to stand with occasional shaking at room temperature for a period of about three weeks. The reagents used were the ordinary laboratory reagents. No attempt was made to regulate the temperature, the solutions being made at room temperature, which ranged around 28°C. A tared 25 ml. graduated flask was filled to the mark with each of the clear supernatant saturated solutions and weighed. The liquid was then transferred with water to a 250 ml. flask, diluted to volume and mixed, and a 50 ml. aliquot was titrated with normal sulfuric acid. From these determinations the following data were obtained:

	KOH in EtOH	KOH in MeOH	NaOH in EtOH	NaOH in MeOH
Density.....	1 04	1 14	0 93	1 01
Grams per 100 ml.....	29 0	40 3	13.6	23.9
Percentage.....	27.9	35 5	14.7	23.6
Normality.....	5 17	7.19	3.40	5.98

Seidell¹, quoting deWaal, states that a saturated solution of potassium hydroxide in ethyl alcohol at 30°C. contains 27.67 per cent of potassium hydroxide. This is in close agreement with the figure given in the table. It is interesting to note that although sodium and potassium hydroxides are almost equally soluble in water at 30°C., potassium hydroxide is much more soluble than is sodium hydroxide in ethyl and methyl alcohols.

¹ Solubilities of Inorganic and Organic Compounds.

THE DETERMINATION OF MANGANESE IN PLANT MATERIALS BY THE PERIODATE METHOD.

By JEHIEL DAVIDSON and RUTH G. CAPEN (Crop Chemistry Laboratory, Bureau of Chemistry and Soils, U. S. Department of Agriculture).

In connection with investigations on the manganese content of plants carried on in this laboratory, the periodate method¹ was used extensively. It was found that this method, which is outlined in the official methods² for plant materials, can be modified advantageously.

The fundamental principle of this method is oxidation by a periodate in an acid solution that must be free from reducing agents. In the determination of manganese in ashed plant materials, the principal reducing agent to be removed is the hydrochloric acid used in dissolving the ash. The procedure given in the official methods calls for boiling with sulfuric and nitric acids in order to expel the hydrochloric acid. Long boiling and repeated additions of nitric acid have been found necessary, however, to remove all traces of hydrochloric acid. To ascertain whether it would not be practicable to omit this troublesome reducing agent, manganese determinations were made by dissolving the ashed plant material in nitric, sulfuric, and phosphoric acids, respectively, instead of in hydrochloric acid, the periodate being added directly to these solutions. The addition of sulfuric acid to the nitric and phosphoric acid solutions was omitted because it proved to be of no advantage. This simple modification made the procedure less troublesome, and resulted in a saving of time. The quantity of chlorine in the ash of plants is generally very small and is well taken care of by the periodate, which is added in excess.

The procedure as outlined in the official methods calls for the addition of ferric nitrate to the standard permanganate solution. This precaution had been suggested by Willard and Greathouse, who worked with materials rich in iron, such as steels and ores, because the color imparted by the iron salts might interfere with the matching of the permanganate solutions. Plant materials, however, contain very little iron, and the small quantities present are generally prevented from imparting color to the solutions by the excess of phosphoric acid in plant ash. It was found quite safe, therefore, to omit the ferric nitrate. On the other hand, the addition of periodate to the standard permanganate solution was found to be very useful, since it serves as an excellent preservative.

A new precaution that was found to be necessary was the guarding of the solutions against alcohol vapors and other volatile reducing substances. The alcohol vapors in the laboratory air were found to pale or even to destroy completely the permanganate color in some cases.

¹ Willard and Greathouse, *J. Am. Chem. Soc.*, 1917, 39: 2366.

² *Methods of Analysis*, A. O. A. C., 1925, 42.

The color is restored on heating, but the determinations must be completed while the solutions are still hot, or the color disappears again.

Results obtained by the procedure as outlined in the official methods and by dissolving the plant ash directly in nitric, sulfuric, and phosphoric acids, are offered for comparison in Table 1.

The materials selected for comparison were two cereal grains high in manganese, two cereal grains low in manganese, and wheatstraw and cornstalks, which are much higher in manganese than the respective grains.

TABLE 1.

Comparison of results of manganese determinations made by the periodate method according to official and modified procedures.*

PLANT MATERIAL	OFFICIAL PROCEDURE	NITRIC ACID	SULFURIC ACID	PHOSPHORIC ACID
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Wheat... ..	0.0085	0.0085	0.0073	0.0085
Rye... ..	0.0091	0.0110	0.0110	0.0110
Rice... ..	0.0049	0.0045	0.0055	0.0061
Corn... ..	0.0013	0.0012	0.0012	0.0015
Wheatstraw... ..	0.0229	0.0259	0.0224	0.0274
Cornstalks... ..	0.0076	0.0076	0.0076	0.0076

* Mn_2O_4 .

The modified procedure is as follows:

Ash the plant materials in platinum dishes in an electric muffle. Add 5 cc. of nitric, sulfuric, and phosphoric acids, respectively, and 20 cc. of distilled water to each dish. Heat the dishes on a steam bath for a few minutes and wash the contents into beakers. Heat the solutions to boiling and keep at this temperature after the addition of 0.3 gram of potassium periodate until the full development of the permanganate color occurs.

A Schreiner colorimeter was used for comparing the solutions. The differences in the results obtained are within the limits of experimental error, but, if anything, better results are obtained by the modified procedure. Of the three acids used, phosphoric acid proved to be most suitable, as it developed the permanganate color in the shortest period of time; sulfuric acid was next in order, and nitric acid was last. In the determinations recorded in the table, the maximum time required for the full development of the permanganate color in the phosphoric acid solutions was only 2-3 minutes from the time the solutions were brought to boiling.

THE IDENTIFICATION OF ATROPINE WITH WAGNER'S REAGENT.

By CHARLES C. FULTON (U. S. Prohibition Service Branch Laboratory, Omaha, Nebr.).

PREVIOUS WORK.

Stephenson, in 1921, stated that Wagner's reagent gives the best microscopic test for atropine, and gave the following description: "The crystals are normally small rods or triangular plates and are formed in great numbers from the amorphous precipitates. * * * This is a satisfactory test, and though the crystals are never large, they are characteristic¹."

However, Glycart and those who collaborated with him in the A. O. A. C. work were not able to recognize the atropine crystals from Stephenson's description². The difficulty is due partly to the small size of the crystals and partly to the very small quantity of atropine usually available for a test, but perhaps it is due chiefly to the varied character of the crystals.

Prior to Stephenson, Putt had given a very different description of the atropine crystals. He stated that oily red drops form first and that these drops then elongate and form small red crystals, nearly uniform in size and often grouped in small clusters or sheaves³. This is a correct description of one type of crystal, but a high concentration of atropine in the test drop is required.

DIFFICULTY OF IDENTIFYING ATROPINE.

The average dose of atropine is only 1/100 grain, and the crystals produced with Wagner's reagent usually require high-power (300-450) for their microscopic examination. The crystals may be rods, grains, or plates—red-brown, red-black, red, orange, or yellow; they vary greatly in size, and a precipitate often contains both red and yellow crystals. The conditions of precipitation must be standardized, if analysts are to obtain comparable results and identify atropine with certainty.

TYPES OF CRYSTALS.

There are at least four distinct types of useful crystals. These are changed from one to another in the following order by increasing the proportion in the test drop of either the atropine or the potassium iodide. The types are as follows:

I—Small red-brown rods.

¹ Some Microchemical Tests for Alkaloids. J. B. Lippincott, 1921.

² *This Journal*, 1928, 11: 354.

³ *J. Ind. Eng. Chem.*, 1912, 4: 508.

II—Small yellow plates.

III—Bicolored precipitate, yellow and dark-red crystals, variously shaped.

IV—Orange-red hexagonal elongated plates, with some dark-red diamonds or triangles and a few yellow cubes or square prisms.

All of the crystals require more iodine than atropine, by weight. With the greatest excess of iodine minute grains are produced, too small for their shape to be distinguished, even under a high-power lens. If the proportion of either atropine or potassium iodide (or both) is increased, the crystals change to type I, then II, III, and IV; then, in addition to the crystals of type IV, pointed red and square yellow prisms are formed, the red predominating; and finally oily red drops, which refuse to crystallize, are formed.

TYPES OF REAGENTS.

The four following type-reagents correspond to the four types of crystals. The reagent is varied in order to obtain each type of crystal over as great a range of concentration of the atropine as possible.

REAGENT	IODINE	POTASSIUM IODIDE	WATER
	<i>gram</i>	<i>grams</i>	<i>cc.</i>
I	1*	2.75*	100
II	1	8	100
III	1	35	100
IV	1	50	100

* Dissolve in 5 cc. of the water and then dilute with the rest of the water.

The first reagent is the most sensitive. An excess of potassium iodide commonly has a slight solvent action on the alkaloid-iodine compound. The most sensitive reagent for general purposes, not merely with atropine, but with most alkaloids, is one saturated with iodine.

PROCEDURE.

Put a drop of atropine solution (about 0.04 cc.) on the microscope slide and add a drop of reagent from a 1 cc. pipet. Mix the reagent evenly through the test drop, using a glass stirring rod, if necessary, but do not allow the test-drop to spread over the slide, because the crystals form best if it is kept within a small compass. (The precipitate is amorphous at first. It soon crystallizes, however, provided the iodine is in excess of the atropine. This is usually evident from a change in the appearance of the precipitate, but in any case it can be observed under the low-power.) As soon as the crystallization is complete or nearly so, apply a cover-glass. Select a part of the precipitate, by observation under low-power, where the crystals are numerous but scattered, and then examine under high-power.

Description of Crystal Types.

TYPE I.

Crystals of type I, small red-brown rods, are best obtained in dilute solutions of atropine, with a reagent containing not more than three

times as much potassium iodide as iodine, by weight. The reagent given, containing 2.75 grams of potassium iodide and 1 gram of iodine, is therefore near the upper limit. It gives best results with solutions of atropine of 1 : 5000–1 : 50,000 concentration. Above 1 : 5000 the precipitate is so heavy that the individual crystals cannot be seen readily. At 1 : 1500 the rods become yellow, and as the concentration of atropine is further increased the crystals become yellow plates (type II). Above 1 : 350 these plates are mixed with crystals of higher types.

The reagent is almost unbelievably sensitive to atropine. A distinct amorphous precipitate is obtained with a 1 : 30,000 solution, but this is by no means the greatest dilution at which crystals can be obtained. Good rods in great number are formed in the 1 : 80,000 solution; in fact, a few characteristic crystals can be obtained with a 1 : 200,000 solution. As the test is made on a single drop, it detects about 0.00025 mg.

Even this sensitivity can be increased a little by increasing the ratio of iodine to potassium iodide in the reagent. This is unnecessary in the case of atropine, and even undesirable, since the crystals are reduced in size. With some other alkaloids, however, or in making general tests for the presence of an alkaloid, it may be desirable to have the most sensitive reagent possible, that is, one saturated with iodine. It requires about 1 gram of potassium iodide, or a little more, to hold 1 gram of iodine in solution in 100 cc. of water.

Reagents of type I give first a red-brown, amorphous precipitate. As crystallization takes place the precipitate turns black, although the individual crystals, as seen under the microscope, may be yellow. The color change is striking and occurs to some extent with reagents of type II also, but not with reagents containing large quantities of potassium iodide.

TYPE II.

Crystals of type II, yellow plates, are best obtained with a reagent containing 4–15 times as much potassium iodide as iodine, by weight. The formula used by Stephenson gives such a reagent. Perhaps the best proportion for atropine is about 8 grams of potassium iodide to 1 gram of iodine. But until more is known regarding the effect on many other alkaloids of varying the composition of the reagent, it might be well to use 10 grams of potassium iodide for this type-reagent and to retain Stephenson's reagent, which contains only 5 grams of potassium iodide.

With reagent II, crystals of type II are obtained with solutions of 1 : 200–1 : 15,000 (the limit of sensitivity), but they are formed best from solutions of 1 : 800–1 : 5000 concentration. At concentrations of 1 : 650–1 : 200, beautiful precipitates, consisting of crystals of type II mixed with those of types III and IV, are obtained.

The yellow plates of type II are usually irregular and are often grouped

in rosettes. When scattered, they often appear rectangular or square. In the mass they seem black, but seen individually under the microscope they are always light-yellow. As the transition to type III is approached (with a 1 : 800 atropine solution) the yellow plates are interspersed with dark crystals which are more or less rod-like.

TYPE III.

Crystals of type III are best obtained with a reagent having 25-35 times as much potassium iodide as iodine. The precipitate is bicolored; the crystals are yellow and dark-red or black, and of various shapes. The yellow crystals are usually rods or short square prisms or cubical grains; the dark crystals vary from irregular black forms, more or less rod-like, to dark-red grains, often diamond shaped or hexagonal. Sometimes the precipitate might be described simply as composed of irregular red and yellow grains.

The formula given contains the maximum amount of potassium iodide for a reagent of this type, and gives good crystals of type IV in the more concentrated solutions. The best crystals of type III are obtained with 1 : 3000-1 : 650 solutions, and the best crystals of type IV, with 1 : 650-1 : 350 solutions.

TYPE IV.

Crystals of type IV are best obtained with a reagent containing 45-65 times as much potassium iodide as iodine. In their most characteristic form they are orange-red to orange elongated hexagonal plates. Along with these plates there are some smaller and compacter crystals, mostly dark-red and triangular or diamond-shaped, while a few are yellow cubes. The triangular dark-red crystal usually has a tooth projecting from one side. One sample of 1/100 grain tablets gave the smaller crystals almost entirely, with only a suggestion of the orange-red plates.

With a reagent of the formula given crystals of type IV are obtained from 1 : 100-1 : 1200 solutions, the best concentration being 1 : 350-1 : 800. The reagent is sensitive to about 1 : 3000, crystals of type III being formed in the more dilute solutions.

TYPE V.

With reagent IV the precipitate from solutions of 1 : 200-1 : 100 concentration first forms oily drops, and crystallization is not so good as from more dilute solutions, but the formation of crystals that may be called type V is interesting. These crystals form as red and yellow prisms, the former usually predominating. The red prisms have pointed ends, the yellow square. Often several radiate from a common center in a small sheaf, and sometimes both colors occur in the same sheaf. These are the crystals described by Putt, obtained, no doubt, with a

reagent having a comparatively small proportion of potassium iodide (the exact formula of his reagent is not given), but with a high ratio of atropine to iodine in the test drop.

UNUSUAL RESULTS.

Pure atropine should give crystals exactly as described. This has been proved by experiments on samples of sulfate, free alkaloid, and tablets, from three different manufacturers. The writer, however, has encountered one sample which gave results somewhat diverse. It consisted of some 1/100 grain tablets of unknown manufacture. Typical rods were obtained with reagent I, but the yellow crystals of type II tended toward small yellow grains interspersed with dark crosses and dark star-like aggregates; reagent III gave small red and yellow grains; and with reagent IV almost all the crystals were dark-red diamonds or triangles, with a few yellow cubes, and in the most concentrated solutions which would crystallize, yellow prisms formed. Extraction of the atropine from these tablets before making up the solutions did not alter the results, so no doubt some alkaloidal impurity was present.

HYOSCYAMINE.

It is worth mentioning that with the four reagents hyoscyamine gives crystals similar to those of its stereo-isomer atropine. These crystals need further study, however.

SUMMARY AND CONCLUSIONS.

(1) Atropine gives at least four different types of useful crystals with Wagner's reagent, depending on the relative proportions of atropine, iodine, and potassium iodide in the test drop.

(2) The types of atropine crystals may be described as follows: I, small, red-brown rods; II, small yellow plates; III, bicolored precipitate, yellow and dark-red crystals; IV, orange-red, hexagonal, elongated plates, the most characteristic and usually the most numerous form, also dark-red, diamond-shaped or triangular grains and a smaller number of yellow cubes.

(3) The crystals generally increase in size in the order given, but all are small and usually require high-power for their microscopic examination. An increase in the proportion of atropine changes the crystals from one type to another in the order given, as does an increase in the proportion of potassium iodide.

(4) A reagent may be prepared to obtain a particular crystal type in atropine solutions of suitable concentration between 1 : 500 and 1 : 5000. When 1 gram of iodine and 100 cc. of water are used, these reagents require potassium iodide as follows: I, 2.75 grams; II, 8 grams; III, 35 grams; IV, 50 grams.

(5) Reagent I is best used with solutions of 1 : 5000–1 : 50,000 concentration; reagent II, with 1 : 800–1 : 5000; reagent III, with 1 : 650–1 : 2000; and reagent IV, with 1 : 200–1 : 800. Care should be taken not to make the test on a solution too concentrated for good results with the reagent used.

(6) Reagents that have the highest ratio of iodine to potassium iodide are the most sensitive. With reagent I characteristic crystals can be obtained from a 1 : 200,000 atropine solution. Even reagent IV is sufficiently sensitive to atropine to be used on a fragment of a 1/100 grain tablet dissolved in a drop of water.

(7) Atropine can be identified, beyond any doubt, by means of the different types of crystals described.

CHEMICAL COMPOSITION OF ALASKAN LICHENS.

By G. C. SPENCER, *Associate Chemist*, and O. F. KRUMBOLTZ, *Junior Chemist* (Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.).

The increasing importance of the reindeer industry in Alaska naturally calls attention to the food supplies upon which this animal must depend. As is well known, lichens constitute the principal forage for reindeer in winter, and even in summer about 15 per cent of the reindeer's food is derived from this source; they have no nutritive qualities which especially adapt them for reindeer food, but they are frequently the only plants which these animals and other ruminants can procure. They thus hold a position of economic importance as a source of sustenance for animals and in a limited way even for human beings.

Owing to the slow growth of lichens, it is highly important to reindeer herders that the proper rotation be maintained in order that the grazing lands be not cropped too closely at any time. This factor is becoming more important as the maximum number of reindeer which can be supported is being approached.

Considered botanically, lichens hold a unique place in the vegetable kingdom, since they are found abundantly distributed in hot as well as in cold climates. Unlike most plants, they derive nourishment chiefly from the atmosphere and only to a limited extent from the soil or other surfaces to which they are attached. They are an important factor in soil formation, however, since they secrete juices which serve to crumble rocks on which they grow.

The most distinctive feature of lichens is their dual nature, whereby a fungus and an alga grow together in a state of symbiosis which permits each species to supply whatever quality or function the other one lacks. Lichens reproduce their kind by fragmentation, by means of soredia,

that is, vegetative reproductive bodies composed of both fungal and algal elements which are budded off from the main body of the thallus (the tough, leathery body of the lichen, formed by fungal hyphae), or by the germination of spores from the fungal symbiont, which, coming in contact with free algal cells, proceed to build up a new individual. The thallus serves to protect the embedded algae, which manufacture carbohydrates like other green plants.

The analytical work recorded in this paper was conducted according to the official methods for feeding stuffs. The "crude fiber", however, gave considerable trouble when filtered, the gummy nature of the residue after the acid and alkaline digestions being such that the usual procedure could not be closely followed. That is, the residues filtered so slowly that they remained in contact with the alkali solution somewhat longer than the 30 minutes which the official method requires. The solutions were soon cooled and diluted so that contact with the hot alkali solution was in no case longer than 35 minutes. The washing, too, was so slow that it may not have been so complete as it otherwise might have been, and in several cases the sample was transferred from the crucible to the cloth filter where it was rewashed, then returned to the Gooch crucible.

As it may be of interest to compare the results in this table with those obtained by analysis of other nongrassy range feeds which serve as emergency rations for cattle and other animals in time of drought, a bibliography is attached.

TABLE 1.
Chemical composition of lichens.

SAMPLE	MOISTURE per cent	FAT per cent	FIBER per cent	PROTEIN* per cent	ASH per cent	N-FREE EXTRACT per cent
1. <i>Cetraria cucullata</i>	12.22	8.70	9.42	1.75	1.27	66.64
2. <i>Cetraria hiascens</i>	14.13	5.23	11.18	2.94	1.90	64.62
3. <i>Cetraria islandica</i>	11.85	2.08	8.53	3.13	1.89	72.49
4. <i>Cetraria nivalis</i>	13.72	4.27	8.26	1.87	2.69	69.19
5. <i>Cladonia alpestris</i>	12.35	1.92	43.98	2.13	2.33	37.29
6. <i>Cladonia amaurocraea celolea</i> ..	12.61	1.55	35.68	1.73	1.48	46.95
7. <i>Cladonia amaurocraea ozyceras</i>	11.88	1.78	33.56	1.50	1.39	49.89
8. <i>Cladonia orispala</i>	12.56	1.34	43.70	2.25	1.85	38.30
9. <i>Cladonia decorticala</i>	13.04	1.14	40.15	4.25	6.27	35.15
10. <i>Cladonia degenerans</i>	12.90	0.76	58.29	3.56	2.21	22.28
11. <i>Cladonia gracilescens</i>	13.27	0.66	40.08	3.06	2.64	40.39
12. <i>Cladonia gracilis dilulala</i> <i>Cladonia bellidiflora hookeri</i> } mixture	12.15	0.89	33.27	3.50	2.92	47.27
13. <i>Cladonia gracilis</i>	12.46	0.85	45.72	2.50	1.79	36.68
14. <i>Cladonia rangiferina</i>	12.83	0.69	47.19	1.75	1.78	35.78
15. <i>Cladonia sylvatica</i> (light form)	12.66	1.45	31.98	1.75	1.81	50.35
16. <i>Cladonia sylvatica</i> (dark form)	13.02	0.57	44.64	1.50	2.05	38.22
17. <i>Cladonia sylvatica sylvestris</i> ..	12.93	1.08	48.92	1.67	1.59	33.81
18. <i>Cladonia uncialis</i>	12.89	1.23	37.26	1.50	1.78	45.34
19. <i>Dactylina arctica</i>	13.12	5.94	8.52	2.81	2.54	67.07
20. <i>Peltigera</i>	13.41	1.12	21.93	17.12	7.91	38.51
21. <i>Stereocaulon tomentosum</i>	12.66	1.94	27.32	5.44	2.09	50.55

* The protein results are averages of duplicate analyses.

SUMMARY.

A table of analytical results on 21 samples of Alaskan lichens is presented.

A brief description of the biological nature of lichens is outlined.

A bibliography of nongrassy range feeds is given.

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SOME OBSERVATIONS ON THE FIEHE TEST¹.

By C. A. GREENLEAF and C. A. BROWNE (Contribution from the Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture).

According to Browne² pure honey that has been boiled will respond to tests for commercial invert sugar that depend on colorimetric reactions of the decomposition products of fructose formed at high temperatures (230°–234°F.). Oxymethyl furfural is generally considered to be the chief decomposition product of fructose that gives color reactions with aniline acetate, resorcinol, and similar reagents. Browne pointed out that boiling or heating honey to high temperatures is, as a rule, most inadvisable since the flavor is seriously injured thereby, but the effect of such heating upon the colorimetric reactions when granulated commercial honey is melted has been in doubt. An opportunity to make some observations on this point was afforded by the kindness of E. L. Sechrist of the Bureau of Entomology, U. S. Department of Agriculture, who furnished a series of samples which he collected in 1928. The source of each sample and the treatment that it had received are shown in Table 1.

Before examining the samples, the three available tests were compared as to sensitivity and ease of interpretation. The resorcin test³ was used. This test is based upon the one proposed by Fiehe⁴, in which the 10 cc. of 1 : 1 honey solution is extracted with ether, the ether extract is filtered into a porcelain dish, and after the ether is expelled at room temperature a few drops of the 1 per cent resorcin solution are added.

¹ Presented before the Division of Agricultural and Food Chemistry at the Columbus meeting of the American Chemical Society and published here by courtesy of *Industrial and Engineering Chemistry*.

² U. S. Dept. Agr. Bur. Chemistry Bull. 110, p. 68.

³ *Methods of Analysis*, A. O. A. C., 1925, p. 201.

⁴ *Z. Nahr. Genussm.*, 1908, 15: 492, and 1908, 16: 75.

The color observed has the same significance as it has in the resorcin test. The Fiehe test is widely used abroad, and its interpretation has provoked much controversy.

Another test is the aniline acetate test proposed in 1907 by Browne¹.

EXPERIMENTAL WORK.

The three tests mentioned were compared by applying them to several natural honeys of known purity and to mixtures of the same honeys with 5 per cent and 10 per cent, respectively, of an invert sugar prepared as directed by Herzfeld².

The following observations were made:

(a) The aniline acetate test discriminates definitely between pure honey and honey containing 10 per cent commercial invert sugar. With 5 per cent commercial invert sugar, the color was barely preceptible. The differences in color were not great enough to recommend the test for determining the effects of heating.

(b) The A. O. A. C. resorcinol test gave results that were positive with 10 per cent of commercial invert sugar, but less evident with 5 per cent. The differences in color were clearly recognizable, however, and this test was adopted in examining the authentic samples.

(c) The Fiehe test is more sensitive than either of the others, but it is more difficult to interpret. In the absence of ether the red condensation product segregates in granules, and the quantity as well as the shade of the colored precipitate must be considered. In contrast to this, in the A. O. A. C. resorcin test the color remains in solution in a fairly definite volume of reagent.

In applying the resorcin test to the authentic honeys, the colors observed were classified as C, colorless; C+, faintly tinged with yellow; Y, yellow; O, orange; P, pink; and R, red. The predominating shade is mentioned first.

In no case did a positive test result from the heating employed.

These results were supplemented by a number of laboratory experiments on honey heated for varying periods of time at constant temperature. A sample of pure alfalfa honey from Colorado was used, and the determinations were made at 15 minute intervals with the A. O. A. C. resorcin test. The final results are given in Table 2.

This sample of honey was of low acidity (equivalent to 12.5 cc. 0.1 *N* sodium hydroxide per 100 grams), and it seemed possible that its resistance to heat might be greater than a honey of higher acidity. To gain some idea of the effect of acidity, therefore, formic acid equivalent to 14.2 cc. of 0.1 *N* alkali per 100 grams was added to a portion to make the total acidity 26.7 cc. per 100 grams. This mixture was heated at

¹ U. S. Dept. Agr. Bur. Chem. Bull. 110, p. 68.

² *Deut. Zuckerind.*, 1900, 31: 1988.

TABLE 1.

Results of tests made on heated and unheated honeys.

NO.	DESCRIPTION	RESORCIN TEST
1	Utah. Unheated; settled in large tanks; quite clear	C +
2	Utah. Unheated; strained through cheese cloth rather crudely. Considerable wax.	C
3	Utah. Heated to about 100°F. in water-jacketed strainer and settled in tanks holding 1250 gallons each (15,000 lbs.). Very clean.	C
4	Idaho. Warmed to about 90°F. in water-jacketed extractor where the honey is exposed in a thin film to moderate heat (water is seldom above 160°F.), and then settled in large tanks.	C
5	Idaho. Heated in large water-jacketed settling tank where the honey, for a part of the time, is exposed to a temperature of 212°F. and after being heated to 140°-160°, is held at about that same temperature overnight. Not very clean.	P
6	Idaho. Honey from capping melter, where it is exposed to 212°F. or less as it flows over a metal pan. In the mass it is not heated above 140°F. and is cooled quickly, remaining hot less than a half hour.	C
7	Idaho. A mixture of No. 6 and honey which was not heated at all, being a sample of honey from capping melter run in with that from the extractor, which cools it at once. It is then strained through cheese-cloth and settled in a tank overnight. Quite clean.	C
9	Idaho. Unheated. Roughly strained and then settled. Not warmed at all. Fairly clean.	C
10	Idaho. Unheated. Settled only. Quite clean.	C
12	Montana. Honey from extractor, not mixed with honey from capping melter.	PO
13	Montana. Heated by coil pipe in bottom of extractor to about 140°F. and then pumped into settling tanks and not further warmed, but is mixed with the honey from capping melter, which is No. 14. Do not know how hot that was heated but it is exposed to steam-heated iron pan and doubtless reaches 160°.	C
14	Montana. Honey from capping melter (see No. 13)	Y
16	Wyoming. Heated in water-jacketed pipe to not above 95° in the mass. Honey from capping melter <i>not</i> mixed with balance of honey.	C
17	Wyoming. Same origin as 16, but this is from a capping melter where the drained cappings are melted and this capping-melter honey kept separate. This is exposed to live steam heat in melting, as it flows over a pan.	C
18	Wyoming. Warmed to about 90°F. in water-jacketed tank; also mixed with honey from warmed draining tank (see No. 19, following).	C
19	Wyoming. Warmed in "heated-grid" uncapping box by steam heat, but not to above 100° in the mass.	C
20	Wyoming. Warmed to not above 100°F. in steam and water-jacketed pipe and settled in 155 gallon tank. Not very clean. Tank too small.	C
21	Montana. Honey from Severin type wax separator, and Root capping melter with additional steam grid. Mass warmed to above 140° but not to 160°. Cooled quickly.	C

Table 1.—Continued.

Results of tests made on heated and unheated honeys.

NO.	DESCRIPTION	RESORCIN TEST
22	Montana. Combination of No. 21 and warmed honey. Warmed in steam-jacketed sump at pump, and by steam coil under settling tank. Mass not warmed above 100°F.	C+
24	Montana. Same style of warming as 22, but honey from another operator, and most probably heated somewhat less and kept warm a shorter time, as there is no steam pipe under settling tank.	C
26	Montana. Mass of honey not warmed at all but mixed with honey from steam-heated capping melter.	C
27	Wyoming. Honey is heated to about 140°F. in open, steam-jacketed trough about 10 feet long; is cooled rather quickly in passing through complicated strainer of under and over type, and then settled in basement (cool). No capping melter honey in this.	C
28	Wyoming. Unheated. Strained and settled. Very clean.....	O (faint)
29	Wyoming. Heated to 155°–160°F. in steam-jacketed pipe and strained through cheese cloth, then settled. No capping melter honey in this. Clean.	C
30	Wyoming. Unheated, except that it is mixed with the honey that comes from capping melter. Strained through cheese cloth but settling tank is too small—not very clean.	C
31	Wyoming. This is a sample of bottled honey. Not heated during extraction, but heated to 155°–160°F. in tank before bottling, and cooled quickly after being bottled. Clear and fine.	C
32	Wyoming. Unheated. Strained through cheese cloth. Very clean...	C
33	Wyoming. Unheated. Gravity settling; clean.....	C
35	Wyoming. Heated to about 140°F. in small tank before bottling. Otherwise not heated.	C
36	Warmed in mass to about 100°F. in pail over gas fire. Strained through cheese cloth, flat. Fairly clean.	C
39	Colorado. Melted comb honey, of a darker original color. Melted in hot-water-heated capping melter. Possibly exposed to near 212° but not above 160° in mass.	OP
40	Montana. Sample from capping melter used in No. 26.....	C
41	Nevada. Not heated, but mixed with honey from Severin capping melter.	C

TABLE 2.

Results of experiments made at constant temperature.

TEMPERATURE	TOTAL TIME	RESORCIN TEST	ANILINE ACETATE TEST	PIEHKE TEST
°F.	hours			
	0	C+		
140	2	C+		
176	2	C+		
208	2	Y-brownish		
221	2	O		
234	1½	PO	+ (faint)	OR

234°F. and compared with the untreated honey, the Fiehe test being used to determine the effects. The results are shown in Table 3.

TABLE 3.

Results of determinations made to ascertain effects of acidity.

TEMPERATURE	TIME	FIEHE TEST	
		Untreated honey	Acidified honey
°F.	minutes		
	0	YO	YO
234	15	YO	O
234	30	YO	RP
234	45	OY	R
234	60	O	R++

In the acidified honey, the reaction after 30 minutes would be classed as positive. The acidified honey darkened more throughout the heating than did the untreated honey.

CONCLUSIONS.

(1) As compared with the aniline acetate test and the A. O. A. C. resorcin test, the Fiehe test for commercial invert sugar in honey is more sensitive but more difficult of interpretation.

(2) Moderate heating of honey is not likely to cause it to give positive reactions for commercial invert sugar.

(3) The destructive effect of heat is increased by high acidity, and a highly acid honey subjected to a high temperature might give positive reactions, especially with the Fiehe test.

A much more efficient modification of the colorimetric resorcin test has been developed by E. K. Nelson of the Food Research Division of the United States Bureau of Chemistry and Soils, who is publishing the following note upon the subject.

NOTE.

Modification of the Fiehe test for the detection of artificial invert sugar in honey¹.

For the Fiehe tests, as carried out by the original directions or as modified by other investigators, a quantity of honey, usually 10 grams, is shaken in a test tube with ether or ground up in a mortar with ether.

In either case a thorough extraction is impossible. The thick honey and ether do not usually mix; if they do, a stubborn and persistent

¹ Contribution from the Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, by E. K. Nelson. Presented before the Division of Agricultural and Food Chemistry at the Columbus meeting of the American Chemical Society, and published here by courtesy of *Industrial and Engineering Chemistry*.

emulsion, which cannot be readily separated, is formed. To avoid this difficulty and to obtain results that can be duplicated and that will represent all the oxymethyl furfural contained in the sample, the following modification of the test was tried and found to be satisfactory:

Dissolve 2 grams of honey in 10 cc. of water, and extract the solution rapidly with ether in a Palkin-Watkins extractor for 30 minutes. Concentrate the ether to about 5 cc. and transfer to a test tube. Add 2 cc. of resorcin reagent, freshly prepared by dissolving 0.2 gram of resorcin in 20 cc. of concentrated hydrochloric acid, and shake the mixture immediately. Note the colors at the end of 5 minutes.

Pure honey, honey mixed with 10 per cent, and honey mixed with 20 per cent of invert sugar were used in testing the method. Pure honey gave an extremely faint pink color; honey containing 10 per cent invert sugar gave a deep pink color; whereas honey containing 20 per cent invert sugar afforded a dark red color.

Re-extraction of the residues for another 30 minutes gave no color in the pure honey sample and only faint colors in the mixtures, showing that 30 minutes' extraction suffices to remove the oxymethyl furfural.

THE ASSAY OF JALAP.

By L. E. WARREN (Drug Research Unit, Food, Drug and Insecticide Administration, Washington, D. C.).

Numerous methods for the assay of jalap are known. After examination of the literature, the six following, which appeared representative of the several types, were selected for preliminary comparative study.

- (1) The U. S. Pharmacopeia X process,
- (2) The Jenkins process (for the assay of podophyllum),
- (3) The washing process elaborated by Dale,
- (4) The Pharmacopeia of the Netherlands V method,
- (5) The French Pharmacopeia method,
- (6) The German Pharmacopeia method.

Each of these methods, except the last, directs that the soluble constituents of the drug be removed by hot alcohol, although the details of the several processes vary somewhat. In the last-named process cold alcohol is used. The U. S. P. X method of extraction and that used in the Jenkins process are essentially identical; consequently these were considered as one. The studies were divided into two phases: (1) processes for extracting the resin, and (2) processes for purifying the resin.

¹ Pharmacopeia of the United States, X, 201 (1926).

² *J. Ind. Eng. Chem.*, 1914, **6**, 671.

³ *Pharm. J.*, 1927, 119, 516.

⁴ Pharmacopeia of the Netherlands, 5, 374 (1926).

⁵ Pharmacopée Française, 365 (1908).

⁶ Deutsches Arzneibuch, Ausgabe, 6, 723 (1926).

U. S. P. X AND JENKINS EXTRACTION PROCESS.

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc. capacity and add 50 cc. of alcohol. Fit the flask with a stopper through which is inserted a glass tube about 2 feet long to act as a condenser and heat the mixture on a steam bath for 3 hours with occasional shaking. Transfer the contents of the flask to a small percolator and percolate with alcohol until about 90 cc. of material has been obtained. Cool the percolate to room temperature and make up the solution to 100 cc. with alcohol.

The Dale process of extraction is essentially as follows:

DALE EXTRACTION PROCESS.

Prepare a 125 cc. separator by packing a pledget of cotton tightly into the stem and a larger pledget into the constriction just above the glass tap on the inside of the separator. (If available, a small percolator provided with a glass tap and a well-fitting stopper may be used.) Place 10 grams of powdered jalap and 50 cc. of alcohol in the separator or percolator. Stopper with a well-fitting cork and place the apparatus in the water oven together with a suitable receptacle to collect the percolate. Open the glass tap and allow the percolation to proceed, forcing the percolate out by the pressure. Continue the percolation with hot alcohol until the drug is exhausted, as indicated when a small portion of the percolate no longer gives a precipitate on being poured into water. If necessary, concentrate the percolate by evaporation on the steam bath and transfer the tincture to a 100 cc. graduated flask by means of small portions of alcohol. Cool if necessary and make up to the mark with cold alcohol. Mix the solution well.

In the Dutch Pharmacopeial method the drug is digested with hot alcohol, and a portion of the filtrate is evaporated. The crude resin is washed once with boiling water, and the residue is dried and weighed; the dried resin is then treated with chloroform to remove certain physiologically inactive constituents, and the insoluble residue is dried and weighed.

In the method of the French Pharmacopeia the drug is heated with alcohol, and a portion of the cooled filtrate is taken. The solution is evaporated, and the residue is treated successively with 15 cc. portions of boiling water until the washings become colorless. The washed resin is then dried and weighed.

The German Pharmacopeia directs that the drug be macerated with cold alcohol and that a portion of the filtrate be taken. The solution is evaporated, and the residue is washed successively with 20 cc. portions of water at 50°C. The washed resin is then dried and weighed.

Each of these three methods involves "aliquot parts", in the sense that this term is usually understood in the assay of crude drugs. In other words, none of these methods is a "complete extraction" process; consequently each is subject to the limitations and inaccuracies inherent in "aliquot part" methods.

In Methods 1, 2, and 3 the processes employed for obtaining the resin differ considerably. They are as follows:

U. S. P. X METHOD.

Transfer 20 cc. of the tincture of jalap, representing 2 grams of jalap, to a separator; add 10 cc. of chloroform and 20 cc. of a saturated solution of potassium citrate (20 grams of potassium citrate dissolved in 12 cc. of distilled water). Shake well during 2 minutes, then set aside for not less than 10 hours or overnight. Draw off and discard the lower aqueous liquid, and decant the alcohol-chloroform solution through a small filter wetted with alcohol-chloroform into a tared flask or beaker. Rinse the separator with a mixture of 10 cc. of alcohol and 5 cc. of chloroform, and pass the rinsing through the filter. Mix the chloroformic liquids, evaporate the solution on a water bath, dry the residue at 100°C., and weigh.

JENKINS ASSAY MODIFIED.

Transfer 10 cc. of the tincture of jalap (equivalent to 1 gram of jalap) to a separator, and add 10 cc. of chloroform and 10 cc. of 0.6 per cent hydrochloric acid. Shake the mixture and allow it to separate; draw off the lower layer into another separator, and repeat the extraction of the liquid in the first separator three times, using 15 cc. of a mixture of one volume of alcohol and two volumes of chloroform each time, and adding these extractions to the extractive in the second separator. Shake the combined extractions with 10 cc. of the 0.6 per cent hydrochloric acid and allow the mixture to separate. Draw off the lower layer into a tared flask and repeat the extraction of the acid liquid three times, using 15 cc. of fresh alcohol-chloroform mixture each time. Evaporate the combined alcohol-chloroform extractions and dry the residue for 20 minutes at 100°C. Repeat the drying in 20-minute periods until the weight becomes constant or begins to increase.

DALE WASHING PROCESS.

Evaporate 50 cc. of the tincture (representing 5 grams of jalap) to dryness in a beaker on the water bath and dry the residue until it is free from alcohol. Place the beaker in a jar of water at 65°C. and add 15 cc. of water at 65°C. Stir the mixture well with a glass rod for 2 minutes to insure thorough washing of the resin. Cool the mixture by placing the beaker in a jar of cold water and decant the wash water through an 11 cm. filter paper. Repeat the washing of the resin with four portions of 15 cc. each of water at 65°C., in each case cooling the mixture and decanting the washings through the filter, as previously described. Dissolve the residue in the beaker in 15 cc. of hot alcohol and pour the solution through the filter, collecting the filtrate in a tared beaker. Use sufficient hot alcohol in small portions to completely transfer the resin to the filter and insure thorough washing of the filter. Evaporate the combined filtrate and washings and dry the residue at 100°C. to constant weight.

Three specimens of jalap were assayed by each of the six methods. The results are given in Table 1.

Obviously in a comparison of methods for the assay of jalap the first question to be considered is which of these gives the most complete extraction of the resinous constituents of the drug. In order to ascertain whether the U. S. P. X method extracts the resin quantitatively, 20 grams of jalap was treated with hot alcohol as directed by the U. S. P. After 200 cc. of percolate had been collected and reserved, percolation with alcohol was continued until a further 100 cc. had been obtained. This solution, which was slightly yellow, was evaporated to dryness on the water bath, and the residue was washed with hot water, as

TABLE 1.
Assays of jalap by several methods.

METHOD	SPECIMEN A		SPECIMEN B		SPECIMEN C	
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
U. S. P. X.....	15.74		14.50		11	34
	15.74		14.60		11	27
			14	30		
Jenkins (modified).....	15.49		14.68		11	01
	15.49		14.45		10	92
					11	21
					11	06
Dale (modified).....	14.42	14.16	13.62		10	67
	14.71		13.60		10	55
	14.27				10	66
	14.28				10	68
Dutch Pharmacopeia V						
Crude Resin.....	14	80	14.49		10	61
	14.73		14.06		10	73
					10	43
Washed Resin.....	13	03	12.73		8	47
	12.80		11.98		9	03
					8.60	
French Pharmacopeia.....	14	35	13.61		10	55
	14.35		13.62		10	82
German Pharmacopeia VI..	14	10	13	17	10	52
	14.17		13	54	10	72

directed in the French Pharmacopeial method. Nearly all the residue was soluble. The insoluble portion was dissolved so far as possible in warm alcohol, the solution was evaporated, and the residue dried and weighed. From 20 grams of original drug a weight of 0.0074 gram was obtained, equivalent to 0.037 per cent. Likewise 50 grams of drug was extracted by the Dale process, and 500 cc. of percolate was reserved. Percolation was continued until a further 100 cc. had been collected. The second percolate was treated like the first. The residues in duplicate determinations weighed 0.0045 gram and 0.0066 gram, respectively, equivalent to 0.009 per cent and 0.013 per cent, average 0.011 per cent. The Dale process extracts a greater quantity of solids from the drug than the U. S. P. method, but this is of no importance since the additional solids are not resin. These tests, together with others not here detailed, warrant the conclusion that the U. S. P. X, the Jenkins and the Dale extraction processes are practically quantitative for the removal of resin from jalap. Since the Dale process is considerably more complicated than that of the U. S. P. X, it is believed that the latter conforms to all of the necessary requirements.

Preliminary tests having indicated that it is probably not necessary to heat the drug in a reflux apparatus with alcohol for as long as 3 hours, as required by the U. S. P. X, a test was undertaken with a view to reducing the time of heating. Twenty grams of the drug were heated

by the U. S. P. X method, but for 1 hour instead of 3 hours. Extraction in the usual way with further percolation after the first 200 cc. of percolate had been received indicated that there was no appreciable activity remaining in the drug. Portions of the first 200 cc. of percolate were assayed and found to contain approximately the same quantity of resin as the percolates from the drug that had been heated longer. The test was repeated, but the heating was continued for 30 minutes only. The results agreed essentially with those obtained from specimens that had been heated for 1 hour and for 3 hours. Accordingly, it was concluded that heating in a reflux apparatus for 30 minutes followed by percolation is sufficient to extract all the resins from the drug.

From an examination of the results in Table 1 it may be seen that the U. S. P. X method and the modified Jenkins process give essentially the same values. These are complete extraction processes, but they are not washing procedures in the sense that that term is usually understood. On the other hand, the Dale method, while a complete extraction process, employs a washing procedure to purify the resin. The results from all of the washing processes agree substantially with each other, but they are lower than by the non-washing methods. However, in the Dutch Pharmacopeia process the resin is directed to be purified by washing with chloroform after a preliminary weighing. In Table 1 the results for this method are given both before and after the chloroform-washing process had been completed. The results obtained after the chloroform washing are lower than those obtained by other methods.

As was noted above, the U. S. P. and the Jenkins processes gave uniformly higher results than were obtained by any of the washing processes, such, for example, as the Dale. Experiments were undertaken to determine whether the fractions represented by the difference between the values from the first two and the third procedures were physiologically active. A quantity of resin was prepared (from specimen C) by the U. S. P. X method of assay. The quantity obtained amounted to 1.0930 grams, equivalent to 10.93 per cent of resin. This resin was then washed by the Dale process. The resin remaining weighed 1.0499 grams, equivalent to 10.50 per cent when calculated to the original drug. The aqueous washings were evaporated to dryness, and the residues were administered to cats. The material was inactive. The same experiment was carried out with the Jenkins process. The resin originally obtained weighed 1.1060 grams, equivalent to 11.06 per cent calculated to the original drug. After being washed the resin weighed 0.9705 gram, equivalent to 9.71 per cent. The aqueous washings were inert when tested on cats, demonstrating that the resin obtained by the U. S. P. assay process and by the Jenkins assay process contains some material which is not found in the resin obtained by the Dale process and which is physiologically inactive.

The German Pharmacopeial method was not further considered because the temperature directed for washing the resin is too low for practical purposes, because too much time (24 hours) is required for macerating the drug, and because the method is an "aliquot-part" procedure. Likewise the method of the Dutch Pharmacopeia was not considered further because it is an "aliquot-part" procedure, because the chloroform-washing gave inconsistent results and because the crude resin is washed but once with hot water. Experience with this and with other methods indicates that one washing is not sufficient to remove quite all the water-soluble substances from the crude resin. However, the error caused by this omission is small. The extraction phase of the French Pharmacopeial method was not further considered because that is an "aliquot-part" procedure. The purification of the resin by the French method, however, was given further study. In order to test the U. S. P., the Jenkins and the Dale washing processes still further, portions of a specimen of jalap (B) were extracted respectively by the U. S. P. method (Method I) and by the Dale process (Method II). The extracts were made up to volume, and aliquot portions of the solution were assayed respectively by the U. S. P. process (Procedure I), the Jenkins process (Procedure II) and by the Dale process (Procedure III). The results obtained by several collaborators are included in Table 2.

TABLE 2.
Assay of a specimen of jalap by several collaborators.

ANALYST	EXTRACTION PROCESS	WASHING PROCESS		
		Procedure I (U. S. P.)	Procedure II (Jenkins)	Procedure III (Dale)
L. E. Warren	U. S. P.	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
		14.50	14.68	13.32
		14.60	14.45	13.19
		14.30		
	Dale	14.76	14.88	13.62
		14.62	14.67	13.60
N. T. Chamberlin Western Reserve University School of Pharmacy	U. S. P.	14.58	13.98	13.78
		15.11	14.32	14.02
	Dale	14.59	13.58	13.55
		15.05	13.93	13.97
L. E. Harris University of Oklahoma School of Pharmacy	U. S. P.	12.34	15.59	13.81
		12.56	15.76	13.84
	Dale	12.31	14.34	13.55
		12.96	14.23	13.66

The results recorded in Table 2 indicate that there is little difference in resin values obtained by the U. S. P. and the Dale processes of extrac-

tion; that the U. S. P. and the Jenkins procedures of assay give essentially the same results; and that the Dale procedure gives the lowest results of any of the three assay procedures tried.

Several dealers were asked to compare the U. S. P. X and the Dale methods of assay, using specimens of drug purchased by them. Only three reports were received. The results are given in Table 3, it being understood, of course, that different samples were used.

TABLE 3.
Comparison of U. S. P. and Dale methods.

COLLABORATOR	SPECIMEN	U. S. P. METHOD	DALE METHOD
		<i>per cent</i>	<i>per cent</i>
Allaire Woodward & Co.....	(1)	8.95	6.20
	(2)	8.95	7.75
Eli Lilly & Co.....	(1)	12.87	12.76
S. B. Penick & Co.....	(1)	8.80	8.18
	(2)	12.72	11.74
	(3)	11.90	10.71

The results are too few to warrant conclusions, but in general they indicate that the U. S. P. method gives higher results than does the Dale method.

Up to this point the experiments made demonstrated that an extraction with alcohol under a reflux apparatus for 30 minutes, followed by percolation with warm alcohol, was sufficient to remove all the resins. The tests demonstrated, further, that one or the other of the washing processes was superior to the U. S. P. or the Jenkins process for obtaining the resin. Since the washing processes of the German and the Dutch Pharmacopeias had been abandoned, it remained to ascertain whether the Dale washing process is superior to that of the French Pharmacopeia. Accordingly 50 grams of the drug was extracted by heating with alcohol for 30 minutes in a reflux apparatus followed by percolation with warm alcohol, the percolate eventually being made up to a volume of 500 cc. with alcohol. Aliquot portions of the solution were then assayed for resin by the Dale washing process and by the French Pharmacopeia washing process. For comparison the results are given in Table 4.

TABLE 4.
Comparison of the Dale and the French washing processes.

WASHING PROCESS	SPECIMEN A	SPECIMEN B	SPECIMEN C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Dale.....	13.99	13.77	10.69
	14.05	13.79	10.62
French Pharmacopeia.....	13.99	13.76	10.37
	13.93	13.81	10.58
			10.61

The findings shown in Table 4 indicate that there is no essential difference in the results obtained from the two processes. The French method is less time consuming than the other.

Although the method of the Dutch Pharmacopeia probably measures the therapeutic properties of jalap more accurately than any other of the six methods tried, it cannot be recommended because in the hands of several collaborators it gave inconsistent results. The chief difficulty appears to be the determination of the chloroform-soluble portion of the resin. Some results obtained by several collaborators on the same specimen of jalap are given in Table 5.

TABLE 5.
Assays of jalap by several collaborators.

COLLABORATOR	SPECIMEN B
	per cent
N. T. C.....	10 5 8 2
W. R. C.....	11.98 11.18
L. E. W.....	12 73 11 98

The chloroform-soluble portion of the resin was tested on cats. It was practically inert as a laxative.

As a result of these studies the method recommended for the assay of jalap is as follows:

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc. capacity and add 50 cc. of alcohol. Fit the flask with a stopper, through which is inserted a glass tube about 2 feet long to act as a condenser, and heat the mixture on a gently simmering steam bath for 30 minutes, shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc. of material has been obtained. Cool the percolate to room temperature and make up the solution to 100 cc. with alcohol.

Evaporate 25 cc. of the tincture prepared as described (representing 2.5 grams of jalap) to dryness on the water bath in a beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 cc. of boiling water and stir the mixture well with a glass rod for 2 minutes to insure thorough washing of the resin. Cool the mixture by placing the container in a jar of cold water and decant the wash water into a 9 cm. filter paper. Repeat the washing of the resin with another 15 cc. portion of boiling water, cooling the mixture after kneading the resin and decanting the washings onto the filter, as described previously. If the second wash water contains much color, it is advisable to wash the resin a third time with boiling water. Dissolve the residue in the container in 15 cc. of warm alcohol and pour the solution onto the filter, collecting the filtrate in a weighed beaker. Use sufficient hot alcohol in small portions to completely transfer the resin to the filter and insure thorough washing of the filter. Evaporate the combined filtrate and washings. Dry the residue at 100°C. to constant weight, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated.

SUMMARY.

Six methods for the assay of jalap were tried. The U. S. P. X and the Jenkins methods gave high results and were discarded. The methods of the Dutch, French and German Pharmacopeias are "aliquot-part"

procedures, and therefore they were rejected. The Dale process requires total extraction, and therefore it is accurate, although it is tedious in execution. It may well be replaced by a shorter process.

A modification of the U. S. P. X extraction process combined with the method of the French Pharmacopeia was devised for obtaining and washing the resin; it is more expeditious than the published procedures and gives equally good results.

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The writer wishes to acknowledge his appreciation to the several pharmaceutical manufacturers and to others connected with schools of pharmacy who have aided in this study by collaborative assays, also to the New York Station of the Food, Drug and Insecticide Administration for furnishing the crude drugs. Thanks are also due to the staff of the Pharmacological Laboratory of the Food, Drug and Insecticide Administration for ascertaining the physiological activity of the several fractions of resin of jalap on cats and to William R. Carter for carrying out a number of the routine determinations and for checking many of the other analyses.

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A STUDY OF THE ACCURACY OF THE GUTZEIT METHOD FOR ARSENIC.¹

By J. R. NELLER (Agricultural Experiment Station, Pullman, Wash.).

The Gutzeit method, which is generally used at the present time for the determination of minute quantities of arsenic, has gone through several modifications and improvements² and has attained a high degree of sensitivity. Owing to this high sensitivity the method tends to produce inaccurate results. In this paper, the writer reviews the findings compiled from work done in connection with spray residue experiments made on fruits during the past three years.

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² *J. Soc. Chem. Ind.*, 1907, 26: 1105; U. S. Dept. Agr. Bur. Chem. Cir. 102 (1912); *This Journal*, 1927, 10: 425, 428; *J. Biol. Chem.*, 1927, 72: 737-43.

Since the method used differs somewhat from the procedure given in the last edition of the official methods¹, the following description is given:

The solution containing the arsenic residues is made up to a volume such that the quantity desired for a determination is contained in an aliquot of from 10 to 20 cc. To this is added 5 cc. of concentrated hydrochloric acid, sufficient water to make a total volume of 60 cc. and 4 cc. of 20 per cent potassium iodide. The mixture is heated in a 100 cc. bottle standing in a water bath at a temperature of 80°–85°C. for 10 minutes. Four drops of stannous chloride (40 grams made up to 100 cc. with concentrated hydrochloric acid) are added, and the sample is cooled to 18°–20°C. in a water bath. The cotton of the upper absorption tube is saturated with a 10 per cent lead acetate solution and the excess is blown out. Cotton placed in the lower absorption tube is saturated with distilled water in a similar way. The stick zinc, cut into pieces about 1 cm. in length, is sensitized by being allowed to react with 10 per cent hydrochloric acid by volume containing 0.1–0.2 per cent of the stannous chloride solution. A certain number of selected pieces of this zinc, 4–6, is added to each evolution bottle. It was found to be important to use equal numbers of similar pieces for each determination. The evolution of hydrogen and arsine, which appears to depend upon the physical structure of the zinc, should be slow, and the proper amount of a given lot needs to be determined to insure stains of sharp definition. The evolution is allowed to proceed from 1½ to 2 hours, during which time the water around the evolution bottle is maintained at from 18° to 20°C. and the air temperature around the absorption tube at 20°–24°C. The length of the stain is fixed by drawing a line across the strip where the stain terminates and then measuring on a graph constructed from the stains obtained from standard solutions, similar to the method of Collins². The stain is measured on both sides of the strip, and an average is obtained therefrom.

Certain changes or refinements of this method were studied with reference to the accuracy obtained and are discussed. In the tables the quantities of arsenic (As_2O_3) obtained are reported to the fourth decimal place in order to make the comparison figures significant; ordinarily arsenic is reported by this method to the third decimal only.

EFFECT OF SIZE OF ALIQUOTS.

When it is considered that the determinations were replicated from 8 to 11 times (Table 1), it is apparent that the probable error of the mean tends to be no greater for the larger than for the smaller sized aliquots, representing as they do a range between 0.02 and 0.09 mg. of arsenic as As_2O_3 . It is apparent from the results given in Table 1 that

¹ *Methods of Analysis*, A. O. A. C., 1925, 171.

² *J. Ind. Eng. Chem.*, 1918, 10: 362.

analytical differences resulting from the use of different sized aliquots from the same solution are generally considerably greater than the probable error for replicated determinations. For the three-spray residue solutions, these differences range from 1.0 to 15.0, with an average of 7.4 per cent. In solutions A and B the larger aliquot gave the larger result, while in solution C the reverse was true.

TABLE 1.
Comparison of results obtained by using aliquots of different volumes.

NO. OF DETER- MINATIONS	SOLUTION	SIZE OF ALIQUOT	MEAN OF ARSENIC AS As_2O_3	P. E. OF MEAN	MEAN OF ARSENIC AS As_2O_3 ON 20 CC. BASIS	VARIATION BETWEEN ALIQUOTS OF DIFFERENT SIZES	VARIATION FROM AVERAGE OF ALL ALIQUOTS PER SOLUTION
						mg.	per cent
11	A	20	0.0392	± 0.0015	0.0392	0.0021*	5.2
11		"	0.0408	± 0.0014	0.0408	0.0004	1.0
8		40	0.0825	± 0.0013	0.0413	0.0005	1.2
11		"	0.0776	± 0.0024	0.0388	0.0020	5.0
Average for solution A.....					0.0403		
8	B	20	0.0269	± 0.0011	0.0269	0.0044	15.0
11		"	0.0292	± 0.0014	0.0292	0.0033	11.2
10		60	0.0939	± 0.0018	0.0313	0.0021	8.0
11		"	0.0905	± 0.0033	0.0302	0.0010	3.4
Average for solution B.....					0.0294		
11	C	10	0.0463	± 0.0012	0.0926	0.0050	5.6
10		"	0.0470	± 0.0023	0.0940	0.0108	12.1
10		20	0.0876	± 0.0013	0.0876	0.0064	7.2
11		"	0.0818	± 0.0010	0.0818	0.0122	13.7
Average for solution C.....					0.0890		
Average variation.....							7.4

* For the solutions A, B and C each of the smaller aliquot determinations is compared with each of the larger.

As shown in Table 1, an aliquot of a given size for each of the solutions A, B, and C is represented by two groups of determinations. In any given group all the determinations were made at the same time in the same water bath and, as stated previously, under standard conditions maintained as carefully as possible. The probable error of the mean of combined duplicate groups is less than the average of the probable errors of the two groups. For example, the probable error of the first two groups of Table 1 is ± 0.0010 mg., as compared with an average probable error of ± 0.0015 mg. for the groups taken separately.

Since the solutions used in Table 1 were obtained by dipping apples in boiling 10 per cent hydrochloric acid for about one minute it was thought that perhaps some substance from the apple other than lead arsenate might be interfering with the arsenic determination. To check this point a solution of lead arsenate powder was made up and analyzed, different sized aliquots being used, as shown in Table 2. Results were much the same as those given in Table 1 with respect to variations among aliquots of different size.

TABLE 2.

Comparison of aliquots of the same volume and of different volumes from a solution of lead arsenate.

NO. OF DETERMINATIONS	SIZE OF ALIQUOTS	MEAN OF ARSENIC AS As_2O_3	P. E. OF MEAN	VARIATION BETWEEN PAIRS OF SETS	VARIATION FROM AVERAGE FOR SAME ALIQUOT	MEAN OF As AS As_2O_3 ON 10 CC. BASIS	VARIATION BETWEEN RESULTS WITH 5 AND 10 CC. ALIQUOTS*	VARIATION FROM AVERAGE OF ALL ALIQUOTS
	cc.	mg.	mg.	mg.	per cent	mg.	mg.	per cent
10	5	0.0279	± 0.0005			0.0558		
10	5	0.0318	± 0.0011	0.0039	12.8	0.0636		
10	5	0.0320	± 0.0010	0.0041	13.4	0.0640		
Average for 5 cc. aliquots		0.0305		0.0002	6.6			
9	10	0.0609	± 0.0022			0.0609	0.0051	8.2
9	10	0.0638	± 0.0011	0.0029	4.5	0.0638	0.0080	12.8
10	10	0.0673	± 0.0018	0.0064	10.0	0.0673	0.0115	18.4
Average for 10 cc. aliquots		0.0640		0.0035	5.5		0.0027	4.3
Average variation					8.8		0.0002	0.3
							0.0037	5.9
							0.0031	5.0
							0.0002	0.3
							0.0033	5.3
Average variation								6.7

* Each 5 cc. is compared with each 10 cc. determination.

Table 2 also shows that the variations in the average results of replicated determinations for aliquots of the same size were practically as great as those for aliquots of different sizes. These findings indicate that variations as high as 8 or 9 per cent may be expected when the Gutzeit method is used even when the results are based upon an average of 10-11 determinations.

THE REACTION TEMPERATURES.

In a consideration of the best conditions for the reaction between arsine and the mercuric bromide impregnated in the strips, other factors are involved besides size of aliquot or quantity of zinc and acid in the evolution chamber. One of these factors is the temperature of the arsine as it reacts with the mercuric bromide. It has been pointed out by Treadwell¹ that the reactions of arsine with mercuric chloride impregnated in the paper strips resulted in a series of compounds of the composition AsH_2HgCl , $\text{AsH}(\text{HgCl})_2$, $\text{As}(\text{HgCl})_3$ and As_2Hg_3 . It is probable that a similar series exists in the case of mercuric bromide, now used, and it is therefore important that the reaction conditions between arsine and mercuric bromide should be carefully controlled. The higher the temperature of the evolution chamber and of the absorption tube the larger will be the proportion of water vapor carried with the

¹ Analytical Chemistry, 2nd. ed., Vol. 1. John Wiley and Sons, New York (1915).

arsine, the result being the presence of more moisture at the seat of the reaction. This should cause a more rapid liberation and fixation of the arsine, but the effect upon the character of the stains was unknown.

This possible effect of temperature was studied by making determinations at 18°–20°, 30°–33°, and 40°–44°C. For temperatures higher than 20°C. the reaction chamber and all but a few centimeters of the absorption tube were immersed in the tank of water of the desired temperature. The results for the 20° and 30° temperature ranges are given in Table 3. The few determinations that were made at 40°–44° are not recorded, as the stains were weak, thin, and spotted.

TABLE 3.
Comparison of sets of determinations made at different temperatures.

NO. OF DETERMINATIONS	SPRAY RESIDUE SOLUTION	TEMPERATURE OF EVOLUTION C°.	MEAN OF As As As ₂ O ₃ mg.	P. E. OF MEAN mg.	VARIATION BETWEEN SETS mg.
12	D	18–20	0.0334	±0.0020	0.0006
14		30–33	0.0340	±0.0016	
11	E	18–20	0.0448	±0.0017	0.0032
11		30–33	0.0480	±0.0010	
9	F	18–20	0.0366	±0.0005	0.0040
8		30–33	0.0406	±0.0006	

As was expected, the stains formed faster at 30°C., but they were not, on the average, appreciably different from those formed at 20°C. It is apparent that the increased rates of evolution and absorption of arsine at the higher temperature compensate each other in the final result, in so far as the length and nature of the stain is concerned, as they do at the lower temperature. The probable errors of the means were about the same in either case, signifying that no appreciable gain in accuracy was to be obtained by conducting the determinations at temperatures above those normally found in a laboratory. Heidenhain¹ states that long, thin stains were obtained at low temperatures and recommends an elevated controlled temperature of 30°C. It is possible that stains produced at temperatures appreciably below 20°C. would be undesirable, but in laboratories where the air temperature is maintained fairly near 20°C. it would appear from the data given in Table 3 that the accuracy of the method is as great at 20°C. as at a temperature maintained at 30°C.

WHEN TO ADD STANNOUS CHLORIDE.

A series of analyses was made to determine whether greater accuracy might be obtained by adding the four drops of stannous chloride before rather than after heating the arsenic solution with potassium iodide. The results (Table 4) show that when hydrochloric acid is substituted

¹ *This Journal*, 1928, 11: 107.

for sulfuric acid, the stannous chloride may be added at either time and not affect the accuracy of the method. However, it would be well to add the stannous chloride at or during the time of cooling of the solution, preparatory to the addition of zinc, because it has a tendency to form sulfur dioxide when sulfuric acid is present.

TABLE 4.
Effect of adding SnCl_2 before and after heating with KI.

NO. OF DETER- MINATIONS	SPRAY RESIDUE SOLUTION	TIME OF ADDING SnCl_2	MEAN OF AS AS As_2O_3 mg.	P. E. OF MEAN mg.	VARIATION BETWEEN SETS mg.
11	C	After	0.0463	± 0.0012	0.0017
11		Before	0.0444	± 0.0013	
8	G	After	0.0330	± 0.0006	0.0010
8		Before	0.0340	± 0.0008	
11	H	After	0.0294	± 0.0007	0.0023
11		Before	0.0317	± 0.0008	

METHOD OF PREPARING SENSITIZED STRIPS.

According to the official method, strips of the proper width are cut and then impregnated with the alcoholic solution of mercuric bromide. To insure an even impregnation by this method it is necessary to draw the strips one by one from the solution and to keep them from touching each other while drying. As the strips dry they tend to curl, and it is necessary to straighten them carefully. It is also necessary to clip off and discard a few centimeters of each strip, because there may be an overload of the mercuric bromide due to capillary movements of the evaporating alcohol.

To avoid these difficulties the paper was impregnated before it was cut into strips. Scott¹ suggested this procedure a number of years ago when, instead of an alcoholic solution of mercuric bromide, the method prescribed a water solution of mercuric chloride to be impregnated into Swedish filter paper. When an alcoholic solution of mercuric bromide is used, special care is necessary to insure an even impregnation of the salt. A sheet of Whatman's cold-pressed drawing paper, 50 cm. long and 28 cm. wide, is loosely rolled and placed in a 1000 cc. graduated glass cylinder. The alcoholic mercuric bromide solution is added in sufficient quantities to cover the paper, which is allowed to soak for 1 hour. The roll is withdrawn with a glass hook and allowed to drain for a few seconds; it is then turned end for end and allowed to drain for a like period. The paper is waved back and forth from two to three times while held at one end to permit uncurling. The other end is then grasped, and the paper is waved back and forth for an equal period of time; the ends are again reversed and this process is continued until the

¹ Standard Methods of Chemical Analysis, 3rd ed., p. 47. Van Nostrand, 1922.

paper looks dry throughout its length. It is next placed upright between glass flasks to air dry completely. During the first part of this period it is well to turn the paper once so that the lower edge changes position with the upper edge. It is considered essential to shift the paper end for end continually, as described above, to insure as even a distribution of the mercuric bromide as possible while the alcohol is evaporating.

Before cutting the paper into strips in a manner proposed by Green¹, a strip 5 cm. wide is cut from each of the edges and discarded. A line is then drawn with a pencil lengthwise across the paper a few centimeters from one edge. A portion of this line on each of the 25 mm. strips serves to mark the end that is kept uppermost in the Gutzeit apparatus. This marking also tends to reduce irregularities, such as unevenness in the thickness of the paper and variations in the width of the strips that might be caused by the knife of the paper cutter. If prepared in this manner the strips remain straight and may be stored in a desiccator or some air-tight vessel.

Analyses were made on arsenical residue solutions for the purpose of comparing the degree of accuracy that might be expected with strips prepared by the two methods. Since the differences (Table 5) in the results obtained when the two types of strips are used are no greater than those obtained when only one type of strip is used, it is apparent that there is equal accuracy with strips carefully prepared by either method.

TABLE 5.

Comparison of strips cut before with those cut after impregnation with HgBr₂.

NO. OF DETERMINATIONS	SPRAY RESIDUE SOLUTION	STRIPS CUT BEFORE AND AFTER IMPREGNATION	MEAN OF AS	P. E. OF MEAN	VARIATION BETWEEN SETS
			AS AS ₂ O ₃ mg.	mg.	mg.
8	I	After	0.0603	±0.0016	0.0039
8		Before	0.0564	±0.0020	
9	J	After	0.0473	±0.0009	0.0034
9		Before	0.0507	±0.0016	
10	K	After	0.0550	±0.0015	0.0030
9		Before	0.0520	±0.0009	

PROBABLE ERRORS OF SINGLE AND REPLICATED DETERMINATIONS.

In connection with the use of the Gutzeit method the question naturally arises as to the number of times a determination should be replicated to insure a sufficiently accurate result. Since two determinations are ordinarily considered sufficient, special attention was given to the accuracy of all the possible pairs of determinations as compared with the average obtained from 8–11 replications. Thus there are 55 different pairs in 11 replicated determinations, 45 in 10, etc. The probable error

¹ *Ind. Eng. Chem.*, 1927, 19: 424.

of a single pair was determined by using the formula for the probable error of a single determination, $P E = 0.6745 \sqrt{\frac{\Sigma d^2}{(n-1)}}$, in which d is the deviation of the average of each pair from the mean and n is the number of pairs. In Table 6 these probable errors are given for the analyses reported in Table 1. If they are considered in terms of percentage of the means it may be observed that they vary from 2.7 to 10.0, or an average of 6.6 per cent. When reference is made to variations between duplicated groups of determinations, as discussed above, it is evident that a result that may be expected from the average of two determinations is sufficiently accurate, as the average error of 6.6 per cent thus obtained is less than 7.4 per cent, the average variation between groups of 8-11 determinations each, as given in Table 1.

TABLE 6.

Probable errors of the mean and of single and duplicate determinations of arsenic calculated from results given in Table 1.

NO. OF DETER- MINA- TIONS	MEAN QUANTITY OF As_2O_3 <i>mg.</i>	P. E. OF MEAN <i>mg.</i>	P. E. OF MEAN IN PER CENT OF MEAN <i>per cent</i>	P. E. OF SINGLE DETER- MINATION <i>mg.</i>	P. E. OF A SINGLE DETERMINA- TION IN PER CENT OF MEAN <i>per cent</i>	P. E. OF PAIRS <i>mg.</i>	P. E. OF PAIRS IN PER CENT OF MEAN <i>per cent</i>
8	0.0219	±.0010	3.7	±.0027	10.0	±.0017	6.3
11	0.0292	±.0014	4.8	±.0032	11.0	±.0025	8.6
11	0.0392	±.0015	3.8	±.0049	12.5	±.0033	8.4
11	0.0408	±.0014	3.5	±.0050	12.3	±.0033	8.1
11	0.0463	±.0012	2.6	±.0044	9.5	±.0028	6.0
10	0.0470	±.0023	4.9	±.0076	16.2	±.0047	10.0
11	0.0776	±.0024	3.1	±.0085	10.9	±.0055	7.1
11	0.0818	±.0010	1.2	±.0030	3.7	±.0022	2.7
8	0.0825	±.0013	1.6	±.0119	14.4	±.0065	7.9
10	0.0876	±.0013	1.5	±.0042	4.8	±.0028	3.2
11	0.0905	±.0033	3.6	±.0080	8.8	±.0057	6.3
10	0.0939	±.0018	1.9	±.0055	5.9	±.0039	4.2
Average.....			3.0		10.0		6.6

PRECAUTIONS.

Since the method given in this paper is based upon a quantitative comparison of stains obtained from solutions of known arsenical content it is important that each set of determinations be made under controlled and standardized conditions in regard to rate of evolution, temperature of stain formation, etc. One very essential point is to keep the cotton in the absorption tubes practically saturated with moisture. It was observed repeatedly that longer and thinner stains resulted when the moisture content of the cotton dropped much below the saturation point. It is preferable to use cotton rather than glass wool because the cotton holds a larger reserve of moisture.

Whenever possible it is advisable to use hydrochloric instead of sulfuric acid in the evolution bottle, thereby avoiding the formation of

hydrogen sulfide. When hydrochloric acid is used it is possible to substitute cotton saturated with water for the roll of lead acetate paper formally recommended for the lower scrubber tube. This substitution serves two purposes: (1) to insure a more constant and complete moistening of the ascending hydrogen and arsine gases, and (2) to decrease the possibility of contaminating the sample with lead acetate. It has been found that a small quantity of lead acetate from the scrubber tube may reduce or completely stop the formation of arsine.

It is also essential that the reduction of arsenate to arsenite be properly accomplished. To insure the completion of this first reduction stage, it was found advisable to keep the acid concentration close to 10 per cent by volume and to heat the solution with the potassium iodide for 10 minutes at 80°–85°C. The reduction to arsenite is thus safely completed and there is little formation of hydrogen sulfide even when sulfuric acid is used instead of hydrochloric acid.

The zinc should be protected from contamination. For instance, traces of mercuric bromide from the sensitized strips may stop the evolution of the arsine and so render the zinc unfit for further use. It is also advisable to complete the analysis as soon as possible after adding the stannous chloride, otherwise sufficient iodine may be again set free to retard the second reduction step to arsine. Because some lots of zinc are much more active than others, the amount of zinc to use should be determined by its activity.

If it is necessary to use sulfuric acid for the preparation of the sample, it is well to add hydrochloric acid to supply the additional acid for the evolution of arsine, thereby reducing the tendency for the formation of hydrogen sulfide.

SUMMARY AND CONCLUSION.

A study was made of the accuracy of the Gutzeit method for the determination of arsenic by using lead arsenate solutions obtained by dipping sprayed apples in boiling 10 per cent hydrochloric acid.

The results obtained when aliquots of different sizes were used varied from 1.0 to 15.0 per cent, or an average of 7.4 per cent, and they were based upon the averages of determinations replicated 8–11 times.

When a solution of lead arsenate powder was used, variations between aliquots of different sizes ranged from 0.3 to 18.4, or an average of 6.7 per cent of the average of all.

Variations in averages obtained when aliquots of the same size were used ranged from 4.5 to 13.4, or an average of 8.8 per cent.

The average probable error in percentage of the mean of all possible pairs obtainable in the various groups of 8–11 determinations each was 6.6 per cent, showing that a duplicate analysis may be expected to give a result within the normal limits of accuracy for this method.

An evolution and absorption temperature of 18°-20°C. was equally as accurate as a temperature of 30°-33°C.

The addition of stannous chloride either before or after heating the solution with potassium iodide did not affect the results obtained. If sulfuric acid is present it is advisable to add the stannous chloride at the time of cooling to avoid the possibility of the formation of sulfur dioxide.

A convenient method of preparing the sensitized strips is described.

Several precautions are listed with reference to the technic of the method.

Since the experimental error of most quantitative methods is less than that obtained with the Gutzeit method, it would be desirable to devise another method for arsenic determinations. The Marsh method may have a greater accuracy, but it is too slow and cumbersome to be satisfactory in most cases.

NEW BOOKS.

Sugar Cane and Its Culture. By F. S. Earle. John Wiley and Sons, Inc., New York, 1928. Price \$4.50. "The results of over twenty years of work and observation in the cane fields of Cuba and Porto Rico" is presented for both the practical field man and technologist. The practical rather than the laboratory research aspect of the subject is stressed. The volume serves to bring together in one source a "fairly comprehensive" view of the sugar-cane situation in all parts of the world.

Standard Yearbook. United States Department of Commerce. Superintendent of Documents, U. S. Government Printing Office, Washington, D. C., 1929. Price \$1.00. "The present volume contains outlines of the activities and accomplishments of not only the National Bureau of Standards and other agencies of the Federal Government and the States, counties and municipalities but also those of technical societies and trade associations, with special emphasis on their accomplishments during the year 1928, their programs for future work, and methods employed by each organization to encourage or facilitate the use of its standards and specifications."

The Bureau of Chemistry and Soils. Institute for Government Research, 1928. By Gustavus A. Weber. The Johns Hopkins Press, Baltimore, Md. Price \$1.50. This monograph is No. 52 of the series prepared by the Institute which "will serve the double purpose of furnishing an essential tool for efficient legislation, administration and popular control, and of laying the basis for critical and constructive work on the part of those upon whom responsibility for such work primarily rests".

Associate Referee on Phenolsulfonate.

Maurice Harris, Food, Drug and Insecticide Administration, Chicago, Ill., has been appointed as Associate Referee on Phenolsulfonate.

As this number of the *Journal* goes to press, we learn with sorrow of the sudden death of Dr. R. W. Balcom, which occurred during the early evening of October 17, 1929. Dr. Balcom served as Chairman of the Board of Editors for many years, and his loss will be particularly felt by its members. During his tenure of office the *Journal* passed through the most trying period of its existence. He gave unstintingly of his energy and efforts for the advancement of the Association of Official Agricultural Chemists, of which he had always been a faithful, loyal, and active member.

An obituary will be presented in an early issue.

BOARD OF EDITORS.

SECOND DAY.

TUESDAY—MORNING SESSION.

REPORT ON EGGS AND EGG PRODUCTS¹.

By J. C. PALMER (U. S. Food, Drug and Insecticide Administration, Seattle, Wash.), *Referee*.

The nature of the subject matter embodied in this report will be along general lines dealing with difficulties involved in the chemical examination of eggs and noodles. The referee was unable to devote any time to the studies recommended in last year's report.

Analytical work was done on old samples of dried egg and noodles for the purpose of showing the effect that aging has upon phosphorus and water-soluble protein. Table 1 shows the amount of work performed, but it is not conclusive, since complete analysis was not made on all samples in 1924. However, it does show that a decided drop in the quantity of lipid phosphoric acid (P_2O_5) in dried eggs occurs on aging. This decrease is noted in the case of one noodle, No. 49559, but it is not shown in No. 49558. The decrease in lipoids is noted in all cases. On only one sample, No. 48590, dried egg, was an opportunity given to note the effect of aging on the water-soluble nitrogen, and here a definite decrease is shown.

Another point of interest brought out by this analytical work relates to the ratios. It has always been considered that a lipid-fat ratio less than unity indicates an old egg product. This is not the case, however, in No. 48590, dried egg. In this instance the ratio is above unity, and furthermore it shows very little change during the last 3 years of aging. The analysis shows the decided drop in lipid P_2O_5 and along with it a drop in lipid. It appears, therefore, that this ratio is not so valuable as it might be in indicating an old egg product. It will be noted that the ratio of lipid/lipid P_2O_5 runs quite constant on all samples analyzed in 1924 and including the noodles analyzed in 1927, but the 1927 analysis of dried egg shows this ratio to be very high. It is truly indicative of the action that has taken place in aging, namely the breaking up of the organic phosphorus into inorganic forms. This ratio appears to be of value in detecting old egg products. It also becomes useful for the purpose of proving the dependability of the alcoholic potassium hydroxide method of saponification of lipoids. The ratio has been calculated on all of Buchanan's work² and on other samples of noodles and eggs. The values obtained run consistently around 40-45 in the hands of different analysts.

¹ Presented by E. M. Bailey.

² *This Journal*, 1924, 7: 407.

The statement that this ratio may be used to prove the efficiency of the potassium hydroxide saponification method and also for the purpose of detecting old egg products may not be apparent. In case an analyst obtains a high lipid/lipoid P_2O_5 ratio on a sample, it may indicate either an old noodle or that he has failed to obtain the correct lipid P_2O_5 value through lack of sufficient fixing agent or improper technic of some sort. A total P_2O_5 value will give the true interpretation of the case involved, and this determination should be made in all cases when the present methods show low lipid P_2O_5 values.

Very few data have been collected to date relative to the quantity of total P_2O_5 (obtained by the aid of a fixing agent) present in various kinds of semolina and flours used in the manufacture of noodles. Examination of flour samples in this laboratory gave the following results:

	TOTAL P_2O_5 per cent
Durum semolina.....	0.353
Durum semolina.....	0.365
50% semolina, 50% hard wheat flour	0.320
Hard wheat flour.....	0.323

Alcoholic potash was used as a fixing agent in these determinations. The analytical work was done by H. A. Reed, Food, Drug and Insecticide Laboratory, Seattle, Wash.

The latter part of Table 1 shows the work done on fixing agents used in connection with total P_2O_5 determinations on egg products. It will be noted that the alcoholic potassium hydroxide gives slightly higher results than the magnesium nitrate-nitric acid reagent in all cases when charred at 550°C. for 30 minutes. (Samples containing potassium hydroxide were charred, not completely ashed; samples containing the magnesium nitrate-nitric acid reagent were completely ashed.) Temperatures of 600°C. and 650°C. were used for ashing for the purpose of ascertaining loss of P_2O_5 or the transfer from the ortho form to the pyro and meta forms of phosphates. None was noted; in fact, the results indicate a higher recovery at higher temperatures in the case of both fixing agents.

Direct ashing at all temperatures was unsatisfactory. This is due no doubt to the change of form from ortho to pyro and meta and consequent failure to precipitate with molybdate solution. An aliquot of No. 49556, direct ashing, which showed a trace of P_2O_5 , was boiled with nitric acid for 20 minutes, and a yield of 1.80 per cent P_2O_5 was obtained. This treatment shows the reversion to the ortho form. No work has been done as yet to show the exact condition under which all the pyro and meta phosphates are transformed into orthophosphate. It appears from the work done that the potassium hydroxide saponification is quite satisfactory when properly applied and charred, even though exposed to

a temperature of $650^{\circ}\text{C}.$, which is much higher than the ordinary analyst would use. In view of this conclusion, it seems unnecessary to take precautions for the prevention of the change from orthophosphates to pyro and meta phosphates.

Another experiment was tried to ascertain the length of time necessary for complete charring when alcoholic potassium hydroxide is used as a fixing agent.

Three portions of egg lipid, *A*, *B*, and *C*, were saponified in the usual manner. *A* was placed in the muffle at $550^{\circ}\text{C}.$ and allowed to remain for 10 minutes after the smoking and combustion of volatile organic matter had ceased; *B* and *C* were allowed to remain in the muffle at $550^{\circ}\text{C}.$ for 1 hour. After removal from the muffle, *B* was boiled with concentrated nitric acid for 5–10 minutes.

The results are as follows:

A – 0.065, *B* – 0.066, *C* – 0.066 grams of P_2O_5 .

It is concluded that complete charring at $550^{\circ}\text{C}.$ is as effective as prolonged heating. An abundance of carbon remained after heating in Sample *A* and a much smaller amount in *B* and *C*.

The boiling with nitric acid failed to increase the yield of P_2O_5 , showing that no pyro and meta phosphates were formed during the heating.

CONCLUSIONS.

Alcoholic potassium hydroxide is a satisfactory fixing agent for the determination of lipid P_2O_5 and total P_2O_5 in eggs and egg products.

The determination of total P_2O_5 , obtained with the aid of alcoholic potassium hydroxide as a fixing agent, is essential for the correct interpretation of the egg content of alimentary pastes.

RECOMMENDATIONS¹.

It is recommended—

(1) That more analytical data be collected on eggs, liquid and dry, and on flours or semolinas used in the manufacture of noodles, special attention being devoted to the amount of total P_2O_5 obtained by using alcoholic potassium hydroxide as a fixing agent. Similar data should be collected on noodles of known egg content made with yolk and also with whole egg.

(2) That collaborative study be given to the following methods: Fat, acid hydrolysis; lipoids; and lipid phosphoric acid.

(3) That study be given to the following methods for detection of decomposition: Acid-soluble phosphoric acid, ammonia nitrogen, and reducing substances as dextrose.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 80.

TABLE 1.

Results of analysis¹.

(Calculations were made on a moisture-free basis and are expressed as percentage.)

Sample Number.....	EGG NOODLES				DRIED EGGS			
	49558		49559		48590		49556	
Date Analyzed.....	1924	1927	1924	1927	1924	1927	1924	1927
Fat (acid hydrolysis).....		4.86		5.01	49.14	48.15		46.04
Lipoids.....	4.94	4.75	5.04	4.56	53.41	51.00	51.38	49.43
Lipoid P ₂ O ₅	0.112	0.113	0.121	0.096	1.185	0.621	1.31	0.671
Total Nitrogen.....		2.72		2.68	7.05	6.97		7.33
Water-soluble Nitrogen.....					3.89	3.27		4.01
Water-soluble Nitrogen Pre- cipitable by 40% Alcohol..		0.559		0.389	2.60	2.30		3.54
Egg Solids (dry basis)*.....	5.15	5.23	5.89	3.82				
Lipoid/Fat.....		0.97		0.91	1.09	1.06		1.07
Lipoid/Lipoid P ₂ O ₅	44.0	42.0	42.0	47.0	45.0	83.0	39.0	73.0
Total P ₂ O ₅ No fixing agent								
Ash temperature 550°C...		0.088		0.055		0.602		0.550
Ash temperature 600°C...			trace†
Total P ₂ O ₅ Alcoholic potassium hydroxide added								
Ash temperature 550°C...		0.450		0.460		2.087		2.044
Ash temperature 600°C...			2.05
Ash temperature 650°C...			2.20
Total P ₂ O ₅ Mg(NO ₃) ₂ , HNO ₃ added								
Ash temperature 550°C...		0.438		0.451		2.044		2.032
Ash temperature 600°C...			2.14
Ash temperature 650°C...			2.14

* *Methods of Analysis*, A. O. A. C., 1925, p. 232.† An aliquot of this sample was boiled for 20 minutes with nitric acid; 1.80 per cent phosphoric acid (P₂O₅) was obtained.

No. 49556, dried egg, was used in the manufacture of noodle samples Nos. 49558-9.

No. 48590, dried egg, was imported from China in 1922.

¹ C. E. Goodrich of the Bureau of Chemistry, Food Control Laboratory, Washington, D. C., and the referee made these determinations in 1927, using methods as outlined in *Methods of Analysis*, A. O. A. C., 1925.

REPORT ON WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL, UNSAPONIFIABLE MATTER, AND ASH IN EGGS AND EGG PRODUCTS.

By SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.), *Associate Referee*.

A sample of dried powdered whole egg was submitted to the collaborators, who were requested to prepare samples of liquid egg for analysis. The directions for analysis sent out with the samples are as follows:

DIRECTIONS FOR ANALYSIS.

Sample A.—Powdered whole egg submitted by referee.

Sample B.—Liquid egg.—Break open 6 fresh shell eggs or warm about 300 grams of borings from a can of frozen whole egg and mix well.

UNSAPONIFIABLE MATTER.

Extract the lipoids according to directions given in J. A. O. A. C., 1925, 8: 602. Determine the unsaponifiable matter in the extracted lipoids by the F. A. C. method (J. A. O. A. C., 1926, 9: 45).

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

This method has been published¹.

ASH.

This method has also been published².

COMMENTS OF COLLABORATORS.

L. H. Bailey (Bureau of Chemistry and Soils, Washington, D. C.).—For some reason I did not secure satisfactory results by Method 2 for nitrogen.

J. H. Bornmann (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.).—I prefer Method 1 for nitrogen. I think it is less work. The only thing I don't like about it is the asbestos in the Kjeldahl flask, but that is a minor point. On Method 2 I didn't get a precipitate with the alcohol (on liquid eggs), though I tried four determinations.

The ash method seems to be satisfactory. Why not put approximately 5 grams of alundum in a dish and weigh accurately, then add the sample and weigh? I believe it is more difficult to add the alundum after the sample is weighed out. I used 90-mesh alundum instead of 60-mesh.

Leonard Feldstein (U. S. Food, Drug and Insecticide Administration, Denver, Colo.).—In the determination of water-soluble nitrogen, Method 2, it was noticed that the directions did not call for the addition of sodium chloride. As it was not known whether this omission was intentional or not, the determinations were made with and without the addition of salt. In the sample of dried egg only a slight turbidity was obtained in the absence of salt, and the result obtained was very low. Apparently salt is necessary for precipitation by Method 2. Neither of the two methods described showed any superiority over the method described in *Methods of Analysis*, A. O. A. C., 1925 (for flour). In Method 1 difficulty was experienced in getting all the precipitate out

¹ *This Journal*, 1929, 12: 56.

² *Ibid.*, 55.

of the nursing bottle into the Kjeldahl flask, a fairly large amount of water being required, and in the subsequent digestion there was a decided tendency to bump and foam and several determinations were lost. It was necessary to watch each digestion carefully, and this seems a waste of time. If all the precipitate is collected on a Hirsch funnel and digested as in the flour method, no foaming is noticeable and very little bumping. The same difficulty in bumping and foaming was experienced in Method 2 in digesting the alcoholic solutions. Method 2 has nothing to recommend it. Why make two nitrogen determinations when one will suffice? A few changes were made in Method 2. Instead of using 20 cc. of 0.1 *N* acid to collect the ammonia in the filtrate from the albumin precipitation, 30 cc. was used, so only one blank was necessary. Another alteration was that 100 cc. of the filtrate from the albumin was used instead of 75 cc.; this obviated any calculation and the larger quantity was believed to give more accurate results.

Some slight changes were made in the method for unsaponifiable matter. Since the quantity of lipoids extracted was very small, only half of the amounts of alcohol and aqueous potassium hydroxide was used for the saponification. The amount of petroleum ether used was reduced to 40 cc. for each extraction of unsaponifiable matter.

L. H. McRoberts (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.).—The petroleum ether available for the determination of unsaponifiable matter contained considerable residue. The blank determination on 350 cc. yielded 0.0183 gram. In the final extraction of the unsaponifiable matter the petroleum ether layers were removed from the extraction cylinder by a blow-off or wash bottle device, often described as the Werner-Schmidt Fat Extraction Apparatus. One and one-half hours' drying was necessary to bring the residue to constant weight.

In the water-soluble nitrogen determination there was no difficulty in obtaining clear filtrates following the extraction with water or after the precipitation with 40 per cent alcohol. Results obtained by Method 2 are greater than those obtained by Method 1.

TABLE 1.

Collaborative results on ash and unsaponifiable matter in powdered and liquid eggs.

COLLABORATOR	ASH		UNSAAPONIFIABLE MATTER	
	Powdered Egg (A)	Liquid Egg (B)	Powdered Egg (A)	Liquid Egg (B)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Alfend	3.46	0.981	2.10	0.62
	3.49	0.977	2.16	0.62
	3.49	0.976		
	3.50	0.984		
Bailey	4.36	1.10	1.96	0.53
	4.70	1.08	2.11	0.63
Bornmann	3.60	0.93		
	3.63	0.96		
Horst	3.43	1.062	2.35	0.70
	3.54	1.064	2.55	0.71
McRoberts	3.56	2.40
	3.63	2.04
Salinger	3.83	1.08	2.03	0.60
	3.96	0.99	2.11	0.64

The procedure outlined in Method 2 is not consistent with that given in Method 1. A 1.2 per cent salt solution is recommended for the precipitation in Method 1, but the addition of the salt is not mentioned in Method 2. This difference was not noticed until the 40 per cent alcohol solution had been allowed to stand overnight. Salt was then added in the Method 2 procedure, and the mixture was allowed to stand about 1 hour before centrifugalizing.

In the ash determination blank determinations are necessary on the alundum. Although the alundum used was ignited at 800°-900°C. previous to its use in the determination, blanks on the quantities used in the analysis, approximately 6 grams, lost 0.0008 gram on ignition at low red heat.

L. A. Salinger (U. S. Food, Drug and Insecticide Administration, Savannah, Ga.).—In extracting liquid eggs with ether I found decantation was not feasible, and therefore arranged to blow it off by means of a siphon and blowing tube, which worked very well. In the analysis of liquid eggs, it would seem desirable that some provision be made in the method to arrange for obtaining the dried product in a more suitable condition for extracting, as by the addition of sand. In the ordinary procedure it is difficult to break up the very tenacious residue obtained sufficiently to get satisfactory action by the solvent.

TABLE 2.

Collaborative results on water-soluble protein-nitrogen precipitable by 40 per cent alcohol in eggs.

COLLABORATOR	POWDERED EGGS (A)		LIQUID EGGS (B)	
	Method 1	Method 2	Method 1	Method 2
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Alfend	3.09	3 14	0 94	0 98
	3.04	3 06	0.96	0 98
Bailey	2.88		0 95	
	2 94		1.17	
Bornmann	3 02	2 32	0.67	
	3 05	2.34	0 60	
Feldstein	3.05	3 14	. . .	
	3.02			
Horst	2.20	2.15	0.97	1.09
	2 20	2.25	1 02	1 18
Salinger	0.73	
			0.72	

DISCUSSION.

With the exception of two results, the agreement among the analysts on ash in powdered eggs is satisfactory. One analyst reports results slightly high and another obtains very high results. The results on liquid eggs must be judged on the agreement between the duplicate results of each analyst, since each collaborator obtained his own sample. The duplicate results check well. The ashing time reported by most of the analysts is longer than was found necessary by the associate referee.

During the year the associate referee made a more thorough study of the use of nickel dishes for ashing eggs and investigated the use of magnesium nitrate as a fixative. The nickel dishes were found to be entirely unsuitable for the determination. Magnesium nitrate was found to be inferior to magnesium acetate as a fixative.

Three of the five analysts whose results on unsaponifiable matter are recorded obtained results that checked closely. Two others obtained somewhat higher results. One of the high results is of doubtful value because of the poor grade of petroleum ether used. Considering the nature of the determination and of the samples, the results are fairly good.

The official method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour requires the filtration of the precipitated proteins through an asbestos mat and the thorough washing of the precipitate. This filtration is difficult and time-consuming when the quantity of precipitate is large, as it is in egg products. Two plans have been suggested to obviate this difficulty. One involves centrifugalizing to pack the precipitate before filtration; the other determines the albumin by difference between the total water-soluble nitrogen and the nitrogen remaining after precipitation of the albumin. These two plans are incorporated in Methods 1 and 2, respectively. Unfortunately, through an oversight, direction for adding 1.2 per cent of sodium chloride to the solution before precipitation in Method 2 was omitted. This resulted in the failure of several analysts to obtain a precipitate by Method 2. Several analysts noted the omission and added salt. The results for Method 1 are satisfactory, only one of the five results being off. The results on Method 2 are not so good. Objection was raised by some of the collaborators to the two nitrogen determinations in Method 2. Although the associate referee has found the indirect method more convenient than Method 1, and as accurate, it is felt that in view of the objections voiced by the majority of collaborators during the past two years against the indirect method, Method 1 should be given preference and work on Method 2 should be dropped.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for ash in dried and liquid eggs described in this report be made tentative.

(2) That the method for unsaponifiable matter in dried and liquid eggs described in this report be made tentative.

(3) That the method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol in dried and liquid eggs, described in this report as Method 1, be made tentative.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 80.

REPORT ON THE DETECTION OF DECOMPOSITION IN EGGS.

By H. I. MACOMBER (U. S. Food, Drug and Insecticide Administration, New York, N. Y.), *Associate Referee*.

Because excellent collaborative results were obtained in 1926 and 1927, it was considered unnecessary to obtain further data on the method for the determination of the acidity of the fat, which was adopted as official (first reading) at the last meeting.

In accordance with the recommendation last year the method for the determination of acid-soluble phosphoric acid has been given further study in an effort to simplify it and increase its accuracy. This method was submitted for collaborative study in 1924, but the results obtained by the different collaborators varied so widely that further study of the method was recommended. The associate referee has been devoting his efforts to the perfection of the acidity of the fat method and securing collaborative data, and therefore has spent but little time on the acid-soluble phosphoric acid until this year. This method, as sent out for collaborative study, consisted briefly of three steps: first, the extraction of the eggs with a 1 to 200 solution of hydrochloric acid and 8 grams of picric acid; second, the digestion of 150 cc. of this extract with 20 cc. of nitric acid and 10 cc. of sulfuric acid; and third, the determination of phosphoric acid (P_2O_5) in the digested residue by precipitation with the official magnesia mixture¹.

This method had been shortened from the original method as given by Pine² by omitting the precipitation as ammonium phosphomolybdate from the determination of P_2O_5 . But even with this step omitted the procedure is still rather long, and it was suggested that a volumetric determination of P_2O_5 might simplify and perhaps increase the accuracy of the method.

This year's work, accordingly, was devoted to a study of a volumetric method with the idea of substituting it for the gravimetric method. In the official volumetric methods recommended for fertilizers no sulfuric acid is used in the digestion of the material; accordingly, an attempt was made to use hydrochloric acid and nitric acid to digest the picric acid solution. This was not successful, owing to the incomplete destruction of organic matter. Precipitation of the ammonium phosphomolybdate direct from the picric acid solution was also tried, but it was unsuccessful, because the precipitate formed when the solution was neutralized did not entirely dissolve on acidifying. An effort was then made to remove the sulfuric acid after digesting the picric acid solution with nitric and sulfuric acids by adding barium nitrate. This method was abandoned on

¹ *This Journal*, 1925, 8: 607.

² *Ibid.*, 1924, 8: 57.

account of the large quantity of barium nitrate necessary and the consequent difficulty in holding down the volume of the solution.

It was found that after digesting the picric acid solution with 10 cc. of sulfuric acid and 20 cc. of nitric acid the determination of P_2O_5 volumetrically gave low results. After the addition of the molybdate solution the ammonium phosphomolybdate precipitated very slowly; however, by diluting the digested residue to 100 cc. and using 50 cc. of this solution for determining P_2O_5 excellent duplicate results were obtained, and in some cases they agreed very closely with the gravimetric results. It was also found that 5 cc. of sulfuric acid and 25 cc. of nitric acid were fully as effective in the destruction of organic matter as the 10–20 cc. combination. These quantities, therefore, were used, and the resulting residue was diluted as above, with very similar or slightly better results. For the determination of P_2O_5 the official volumetric method was used, except that before adding ammonium hydroxide 10 cc. of strong nitric acid and about 1 cc. of 10 per cent ferric chloride solution were added to the acid solution. Strong ammonium hydroxide was added until a precipitate formed, and dilute nitric acid (1 + 3) was added slowly until this precipitate just dissolved on being stirred vigorously. Without the ferric chloride no precipitate would form in the solution.

The following results, expressed in milligrams of P_2O_5 per 100 grams of sample on the dry basis, were obtained with three different lots of dried yolk and one lot of dried whole egg:

	GRAVIMETRIC	VOLUMETRIC
Whole egg.	(1) 177.6, 173.7; Ave. 175.7 (2) 171.3, 184.7; Ave. 178.0	198.3, 197.8; Ave. 198.1
Yolk No. 18. . . .	(1) 229.0, 230.5; Ave. 229.8 (2) 181.5, 223.4; Ave. 202.5	(1) 234.6, 233.5; Ave. 234.1 (2) 235.4, 235.9; Ave. 235.7
Yolk No. 19. . . .	120.8, 119.2; Ave. 120.0	(1) 130.9, 130.5, 130.3; Ave. 130.6 (2) 129.8, 129.1, 130.5; Ave. 129.8
Imported yolk. . .	206.2, 200.0; Ave. 203.1	204.0, 206.2; Ave. 205.1

It should be noted that all the volumetric results were obtained by digestion with 5 or 10 cc. of sulfuric acid and 25 or 20 cc. of nitric acid followed by dilution of the remaining solution to 100 cc. and the determination of P_2O_5 in 50 cc. of this solution. The gravimetric results were obtained by using the method outlined at the beginning of this paper.

These figures would seem to indicate that the volumetric method is more accurate than the gravimetric. In every case the duplicates on the same determination are very close, and where there is more than one determination the two determinations agree very closely. In the case of yolk No. 18 the averages of two different determinations are 234.1 mg. and 235.7 mg. and for yolk No. 19 the averages are 130.6 mg. and 129.8

mg. However, in only two cases out of the six given do the volumetric and gravimetric results check closely. Moreover, in two instances the duplicates in two of the gravimetric determinations do not agree. In one case there is a difference of 13 mg. and in the other 42 mg.

Thinking that the single precipitation gravimetric method might be at fault, the referee made several determinations by the official gravimetric method. The results were as follows: Whole egg 187.9, 186.3; Ave. 187.1, which is about half way between the 178.0 of the gravimetric method and the 198.1 of the volumetric method, and checks neither very closely.

Yolk No. 18. . . (1) 229.0, 241.4; Ave. 235.2.
(2) 229.0, 228.1, 226.5; Ave. 227.9.

These averages check very closely the volumetric averages 234.1 and 235.7 and one of the gravimetric averages, 229.8. In the first determination, however, the duplicates do not check very closely, being 12.4 mg. apart.

For lack of material only a single determination was made on Yolk No. 19, 127.9, which checks closely the volumetric averages 130.6 and 129.8, but is not very close to the gravimetric average, 120.0.

Judging from these results the referee feels that it is necessary to secure more data on both the volumetric and gravimetric methods before either one is sent out for collaborative study.

No study was made this year of the methods for determining ammonia nitrogen and reducing substances as dextrose.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for the determination of acidity of the fat, as described in the 1926 report², be adopted as official (final reading).

(2) That the method for the determination of acid-soluble phosphoric acid be given further study, with the object of securing more information as to the comparative merits of the volumetric and gravimetric methods.

(3) That a study be made of the methods for determining ammonia nitrogen and reducing substances as dextrose, with the object of securing collaborative data.

No separate report on total solids, fat, lipoids, and lipoid phosphoric acid (P_2O_5) was given by the associate referee. See Report on Eggs and Egg Products by the General Referee, who was also Associate Referee on this subject.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 80.

² *This Journal*, 1927, 10: 50.

REPORT ON FOOD PRESERVATIVES.

By WYATT W. RANDALL (State of Maryland Department of Health, Baltimore, Md.), *Referee*.

The collaborative work done upon the determination of preservatives since the last meeting of the association has been so meager that no detailed report will be given.

In an effort to fix upon a method for the determination of saccharin in food products more satisfactory than that in *Methods of Analysis* and to devise methods for the separation and determination of saccharin and sodium benzoate when occurring together in such products, the referee prepared a scheme of analysis and issued samples to a number of chemists. Unfortunately, for one reason or another, some of these analysts were unable to carry the work through. J. Hoffmann and W. J. Johnson of the Minnesota State Dairy and Food Laboratory reported a series of determinations made by them in accordance with the scheme suggested. W. H. Schulze of the State of Maryland Department of Health began a similar study, but by reason of a prolonged illness was unable to complete it. It was deemed wise, therefore, to postpone a full report upon this work until next year.

RECOMMENDATIONS¹.

It is recommended—

(1) That during the coming year the search for a method for the determination of saccharin in food products, which shall be more expeditious than the present official method and shall not involve the destruction of the saccharin, be continued.

(2) That the effort be continued to devise a method for the separation and determination of saccharin and sodium benzoate, respectively, when both are present in a food product.

REPORT ON COLORING MATTERS IN FOODS.

By C. F. JABLONSKI (Food, Drug and Insecticide Administration, New York, N. Y.), *Referee*.

It was recommended—

(I) That further study be devoted to the quantitative separation of amaranth from tartrazine, and that more experimental data be collected.

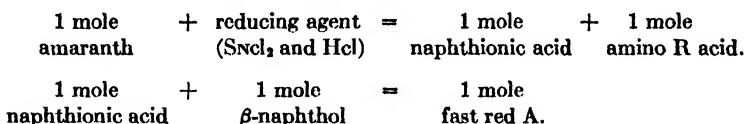
(II) That work be undertaken to obtain a chemical method for separation and quantitative estimation of fast green FCF (recently adopted) from light green SF yellowish and guinea green B and other permitted dyes.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 81.

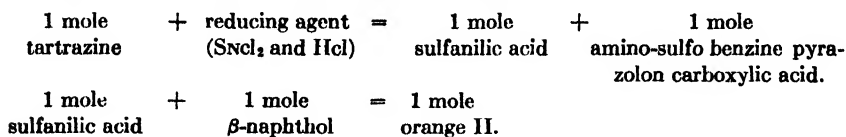
I. *Theoretical Part.*

The reduction and diazotization method proposed by the referee in last year's report was further studied and modified, and it now appears to give quantitative results.

Amaranth and tartrazine are azo dyes, and as such are reduced by stannous chloride in hydrochloric acid solution. The reduction products formed consist initially of two amines from each dye. However, in each case one of these amines is unstable, and therefore it is not subsequently diazotized or coupled. The active components consist of naphthionic acid from amaranth and sulfanilic acid from tartrazine. These amines are produced quantitatively mole for mole; a mole of naphthionic acid represents a mole of amaranth, while a mole of sulfanilic acid represents a mole of tartrazine. By diazotizing naphthionic acid and coupling with β -naphthol, fast red A is produced, a red coloring matter similar in tint to amaranth but of a quite different solubility; while by diazotizing sulfanilic acid and coupling with β -naphthol, orange II is produced, an orange coloring matter unlike tartrazine in shade and solubility. When these dyes are extracted by immiscible solvents, they can readily be separated from each other and estimated volumetrically with standard titanium trichloride. The volume of titanium trichloride required to reduce fast red A represents amaranth, and that required to reduce orange II represents tartrazine. Thus,



Amino R acid decomposes into 1-2 dioxy naphthalene 3-6 disulfo acid, and as such does not enter into further reaction.



The pyrazolon is stable under the proposed treatment, and therefore it does not enter further reactions.

METHOD.

REAGENTS.

Stannous chloride, 40 per cent.—40.0 grams of stannous chloride in concentrated hydrochloric acid, q. s. 100 cc.

Ammonia sodium chloride.—2.5 grams sodium chloride; 4.0 cc. of ammonium hydroxide, sp. gr. 0.9; water q. s. 100 cc.

Copper sulfate, 10 per cent.—10.0 grams of copper sulfate; water q. s. 100 cc.

Sodium nitrile, 10 per cent.—10.0 grams of sodium nitrite; water q. s. 100 cc.

β -naphthol, 1 per cent.—1.0 gram β -naphthol; dilute alcohol q. s. 100 cc.

Sodium carbonate, 2 N.—Sodium carbonate anhydrous, 106 grams; water q. s. 1 liter.

Hydrochloric acid, 0.25 N.—10 cc. of concentrated hydrochloric acid; water q. s. 500 cc.

Hydrochloric acid, 1/128 N.—3 cc. of concentrated hydrochloric acid; water q. s. 4 liters.

An estimate of total color should be made by titrating with 0.1 *N* titanium trichloride. A convenient charge requires about 10 cc.

The quantity of mixed dyes should be the equivalent of 0.10–0.15 gram (1). All determinations must be made in duplicate.

REDUCTION AND DIAZOTIZATION.

Adjust the dye solution to a volume of 50 cc. with water in a 250 cc. centrifuge bottle. Carefully add 3.0 cc. of the accurately measured stannous chloride solution and place the bottle in a water bath, maintaining the temperature of the contents between 50°–60°C. for 3 hours (2). (The solution should become colorless.) Remove the bottle from the bath, allow it to cool to room temperature (20°C.), and add accurately 3.0 cc. of ammonia solution (sp. gr. 0.9). (The mixture should become slightly alkaline to litmus paper.) Mix well, centrifuge, and decant through a 15 cm. quantitative filter into a 400 cc. beaker which has been placed in ice water. Into the beaker measure 15.5 cc. of concentrated hydrochloric acid (sp. gr. 1.18) and 0.2 cc. of copper sulfate solution (3). Wash the magma in the bottle thrice with 50 cc. portions of the ammoniacal sodium chloride solution (4), mix and centrifuge each time, and decant through the filter. (The filtrate in the beaker should measure approximately 200 cc.) Cool the contents of the beaker to 6°C. and add slowly 1 cc. of 10 per cent sodium nitrite solution. Keep the temperature between 7° and 10°C. for 2 hours, testing with starch iodide paper (5) about every half hour. (Under ordinary conditions this quantity of sodium nitrite is found sufficient.) Prepare a solution of 12.0 cc. of β -naphthol (6) in 100 cc. 2 *N* sodium carbonate in a large beaker (1 liter) and cool same to about 15°C. Into this alkaline β -naphthol solution pour gradually the diazo solution, stirring vigorously. Rinse the beaker with some of the dye solution, and lastly rinse with 25 cc. of alcohol and add to the dye solution (1). Transfer the contents of the beaker to a 500 cc. Erlenmeyer flask and allow to stand, preferably overnight, at room temperature.

SEPARATION AND ESTIMATION OF FAST RED A AND ORANGE II.

Use 5 separatory funnels of about 250 cc. capacity and measure into each 50 cc. of amyl alcohol. Add to the first funnel 50 cc. of the mixed dye solution. Shake vigorously for 2 minutes, wait until a sharp separation occurs, draw off the lower layer and pass successively through the other four funnels, and lastly discard the lower layer. Repeat the procedure with 50 cc. portions until the entire dye solution is extracted. Next wash the amyl alcohol extract with four 50 cc. portions of 0.25 *N* hydrochloric acid, shaking vigorously and passing successively through the whole series of funnels. Discard the washings. Dilute each amyl alcohol extract with an equal volume (50 cc.) of petroleum spirit (ligroin) and extract out the orange dye with 50 cc. portions of 1/128 *N* hydrochloric acid, shaking vigorously and passing the lower extract through the entire series of funnels. Collect the orange II in a liter casserole. Continue washing the amyl alcohol until all orange dye is removed. (It will be noted that the first funnel will readily give up the orange dye inasmuch as the red is not readily washed out with that acid concentration.) Continue the acid extractions until each funnel will yield no more orange dye. (Care is advisable in order to detect the phase where all

the orange is removed. But even though the inexperienced manipulator should wash out small quantities of the red dye, the error introduced in the final titration is negligible and could be considered as an experimental error.) Add an excess of ammonia solution (10 cc.) to the casserole containing the orange dye and evaporate carefully to dryness on the steam bath, avoiding spattering. Now extract the amyl alcohol—petroleum spirit (ligroin) solution containing the red dye, using 50 cc. portions of water, until all the dye is extracted. If emulsions should persist during these operations, add 10 cc. of ethyl alcohol to break them. Collect the red dye in another liter casserole; make alkaline with ammonia solution (10 cc.); and evaporate carefully on the steam bath to dryness, avoiding spattering. Dissolve the coloring matter from the casserole with hot water and transfer to a 300 cc. Erlenmeyer flask. Rinse the casserole with 10 cc. of ethyl alcohol and add to the flask the rinsings and enough water to make the volume 100–125 cc.; also add 10.0 grams of sodium bitartrate and 1 drop of 2 per cent light green SF yellowish solution (indicator). Boil vigorously, pass in a rapid stream of carbon dioxide, and titrate with standard titanium trichloride until the green color is visible. Note reading and add another drop of the standard solution to destroy the green color.

The number of cc. of standard solution required to reduce fast red A is equivalent to the amount of amaranth originally present times the factor 1.058 (7) ($\text{cc.} \times 0.01511 \times 1.058$).

The number of cc. of standard solution required to reduce the orange II is equivalent to the amount of tartrazine originally present ($\text{cc.} \times 0.01336$).

NOTES.

(1) If a larger charge of mixed dyes is used, the number of funnels must be proportionally increased.

(2) If the acid concentration or temperature is materially increased, additional sulfanilic acid may be produced from the pyrazolon compound.

(3) Copper sulfate apparently acts as a catalyser¹.

(4) This solution prevents the formation of colloidal solutions of tin and it also facilitates the solution of the amines.

(5) Make a starch paste by triturating 10 parts of starch with 200 parts of water, bring to a boil, and add 1 part of potassium iodide. Impregnate strips of white filter paper with this solution, dry, and preserve carefully.

(6) Alpha-naphthol has also been used for coupling, the dyes formed being fast brown N and orange I. Although their solubility ratios are farther apart, their respective tint in dilute solutions is quite similar, which makes it difficult to separate them.

(7) This value has been experimentally determined. Since it was found impossible to estimate fast red A in an acid solution, the bitartrate buffer salt had to be used. It represents the ratio of $\frac{\text{acid titration}}{\text{sodium bitartrate titration}}$.

EXPERIMENTAL RESULTS.

The results tabulated below were made possible only through the efforts and cooperation of C. F. Bickford of the New York laboratory.

¹ J. C. Cain. *The Chemistry of Diazo Compounds*, 1908 ed., p. 16.

Mixture of amaranth and tartrazine.

EXP. NO.	AMARANTH			TARTRAZINE			TOTAL DYE		
	Taken	Found	Error	Taken	Found	Error	Taken	Found	Error
1	0.0445	0.0463	+0.0018	0.0857	0.0829	-0.0028	0.1302	0.1292	-0.0010
2	0.0833	0.0859	+0.0026	0.0411	0.0391	-0.0020	0.1244	0.1250	+0.0006
3	0.0417	0.0426	+0.0009	0.0686	0.0697	+0.0009	0.1103	0.1123	+0.0020
4	0.0014	0.0012	-0.0002	0.1303	0.1302	-0.0001	0.1317	0.1314	-0.0003
5	0.1200	0.1208	+0.0008	0.1200	0.1208	+0.0008
6	0.1056	0.1011	-0.0045	0.1056	0.1011	-0.0045
7	0.0764	0.0729	-0.0035	0.0171	0.0206	+0.0035	0.0935	0.0935	=0.0000
8	0.0653	0.0636	-0.0017	0.0086	0.0108	+0.0022	0.0739	0.0744	+0.0005
9	0.0069	0.0069	=0.0000	0.0856	0.0872	+0.0012	0.0925	0.0941	+0.0016
10	0.0486	0.0454	-0.0034	0.0600	0.0640	+0.0040	0.1086	0.1094	+0.0008
11	0.0486	0.0477	-0.0009	0.0771	0.0775	+0.0004	0.1257	0.1252	-0.0005
Ave.	0.05223	0.05136	-0.00087 -1.7%	0.06941	0.07028	+0.00087 +1.25%	0.12164	0.12164	=0.0000

CRITICISM.

(a) To tabulate each result in percentage figures is really not significant, since small errors in small charges are proportionally magnified, but the average of these determinations for amaranth or tartrazine, or the total dye, gives quantitative results.

(b) A number of substances, as for example large percentages of (1) sucrose, (2) glycerine, and (3) indigo carmine, were found to interfere with the reduction, diazotization and coupling, and consequently they caused lower results.

(1) A compound mixture containing 10.0 grams of sucrose and 0.1252 gram of mixed dyes (about 1.2 per cent of total), upon reduction, diazotization and coupling gave results for amaranth equivalent to about 70 per cent and for tartrazine equivalent to about 80 per cent. It was noted that during the reduction the resultant solution was not colorless, but of a light yellow shade. Apparently the sucrose was reduced to invert sugar and partly caramelized, which prevented complete reduction and therefore was the cause of an incomplete yield. It is believed that this error can be eliminated by extracting the mixed dyes from an acid solution (by amyl alcohol), removing the dye from the solvent with water, evaporating to dryness (adding excess of ammonia to prevent charring), and following the procedure as above. A number of experiments carried out in this manner gave quantitative results.

(2) A mixture containing 13.0 grams of glycerine and 0.1252 gram of mixed dyes (about 0.9 per cent of total), upon reduction, diazotization and coupling gave results for amaranth equivalent to about 80 per cent and for tartrazine equivalent to about 90 per cent. The reduction solution was not entirely colorless; it was a faint yellow. Lack of time prevented additional experiments.

(3) A mixture containing 0.50 gram of indigo carmine and 0.1252 gram of mixed dyes (about 20 per cent of total), upon reduction, diazotization and coupling gave results for amaranth equivalent to about 70 per cent and for tartrazine equivalent to about 75 per cent. During the reduction it was noted that a heavy precipitate was formed (presumably due to isatine compounds), and the reduced solution was not colorless but a deep brown. Difficulty was further experienced in obtaining a clear filtrate, as the quantity of sodium nitrite solution necessary for diazotization also had to be increased. Repeated experiments gave similar results. It appears that the presence of large quantities of indigo carmine in a mixture with amaranth and tartrazine does not permit of a quantitative estimation, and therefore presents a problem which should be further studied, since color mixtures containing these dyes are quite numerous.

(c) A mixture containing 10.0 grams of sodium chloride, C. P., and 0.1252 gram of mixed dyes (about 1.2 per cent of total), upon reduction, diazotization and coupling gave results close to the theory. The reduction solution was colorless, and a shorter period of time was required to obtain complete reduction. Additional experiments checked these findings. It may therefore be safely said that sodium chloride does not inhibit the reduction.

II. *Investigational Work.*

The amount of investigational work devoted to this problem was rather limited, and the result therefore must be considered of a preliminary nature. Several interesting reactions have been noted which are not claimed to give quantitative results, but with perhaps some additional modification may be developed into a satisfactory quantitative method. However, as a qualitative test they are very striking.

To test the sensitivity of one of these reactions just referred to, two 10 cc. solutions of the new green FCF were made with two different concentrations (0.01 per cent and 0.001 per cent); they yielded, respectively, 0.001 and 0.0001 gram of the dye. They were put into two test tubes and treated with a succession of different reagents, each reagent being added separately to the mixture already in the test tube.

The following changes were observed:

REAGENT	0.01 per cent solution	0.001 per cent solution
2 cc. concentrated hydrochloric acid	greenish yellow	yellow
2 cc. saturated bromine water	yellow, turning greenish	yellow
2 cc. saturated hydrazine sulfate solution	bluish green	slightly blue
3 cc. concentrated ammonia water	pink	colorless
3 cc. glacial acetic acid	deep violet blue	blue

Light green SF yellowish and guinea green B are destroyed by the bromine treatment and become colorless with subsequent treatment with hydrazine sulfate, ammonia water, and acetic acid. Therefore new green FCF can be detected in quantities between 0.1 and 1.0 mg. in either of these dyes.

Another reaction of importance noted by Bickford is obtained by treating a solution of new green FCF equivalent to 0.1–1.0 mg. with a few drops (0.2 cc.) of concentrated hydrochloric acid, adding 1.0 cc. of ethyl nitrite solution, and heating the same on the steam bath. The color of the solution changes into a strong reddish blue shade. Light green SF yellowish and guinea green B are destroyed under similar treatment.

The products of neither of these dye reactions were isolated, and the nature of the reaction has not been established, but it appears that the first reaction is of an oxidizing nature, while the other involves a condensation product.

Therefore additional experimental work should be devoted to these reactions of new green FCF.

RECOMMENDATIONS¹.

It is recommended—

(1) That the referee be requested to send samples of mixtures of amaranth and tartrazine to various collaborators to test out the accuracy of the method outlined in this report.

(2) That further study be devoted to the elimination or conversion of interfering substances when determining amaranth and tartrazine.

(3) That additional work be undertaken to obtain a quantitative estimation of new green FCF and its separation from other permitted dyes.

REPORT ON METALS IN FOODS.

By W. F. CLARKE (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

ARSENIC.

Because of the numerous changes in details that are being studied in connection with the examination of sprayed fruits, it has seemed premature to carry out any collaborative work on arsenic. The referee made some study of the Western District Method² and obtained results which indicate that it is as accurate as other methods and also simpler and quicker.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 81.

² Food, Drug and Insecticide Administration, U. S. Dept. of Agriculture.

LEAD.

The thiocyanate-colorimetric method for lead in the presence of aluminum, copper, iron, tin, and zinc has been studied, with results which, in connection with previous work, indicate that this procedure may be at least as good as the acetate method.

TIN.

In various iodometric methods for tin, it has been found necessary to standardize the iodine by means of a standard tin solution, because the use of iodine standardized against thiosulfate led to inconsistent results. It is supposed that the reaction, under the conditions existing, is not as represented by the equation usually assigned. A study of this reaction and of the results obtained indicate that this difficulty is probably due to the high acidity. Correction of this factor has given results that encourage the belief that concordance may be established by the use of strong sodium hydroxide solution.

RECOMMENDATIONS¹.

It is recommended—

(1) That modifications of the Gutzeit method and volumetric and gravimetric methods for arsenic be studied collaboratively.

(2) That the thiocyanate-colorimetric and the acetate-colorimetric methods for lead be studied collaboratively.

(3) That the correction of the conditions in the iodine volumetric methods for tin, especially in connection with Baker-Sellers and the zinc-iron precipitation methods, be studied collaboratively.

REPORT ON FRUITS AND FRUIT PRODUCTS.

By H. J. WICHMANN (U. S. Food, Drug and Insecticide Administration, San Francisco, Calif.), *Referee*.

At the last meeting of the association it was recommended that collaborative work be done on the determination of the major bases in plant ashes, particularly fruit ashes, by the methods recommended by the referee. This work was done, but only the results from the San Francisco Station are reported by the associate referee. This is unfortunate and rather disheartening, since it indicates a lack of interest. The few results reported are excellent in the referee's opinion, but not numerous enough to form the basis for a recommendation for the adoption of the methods as tentative. The referee, therefore, urges that the collaborative feature be repeated and hopes that other analysts besides those from

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 81.

the San Francisco Station take part in it. Ash results are important in the interpretation of fruit and fruit products analyses, particularly so in the case of citrus fruits.

Colorimetric methods for small quantities of iron and aluminum are available, but since these elements, if present in appreciable quantities, must be removed previous to the determination of the alkaline earths, it is recommended that the associate referee take up as soon as possible the problem of combining the necessary separation of iron and aluminum with their determination as phosphates.

No report other than a rather pessimistic letter was received from the Associate Referee on Fruit Acids. The referee, therefore, can only recommend that the search for a method for the determination of malic acid (active or inactive) be continued. The referee also believes that the associate referee should submit the Hartmann modifications of the citric acid method to collaborative test in preparation for their adoption as tentative methods.

In reports of previous referees the problem of the determination of solids in sucrose solutions containing organic acids has been studied. It has been shown that these solids are too high by 5 per cent of the sucrose inverted during the drying. Kathryn Breen of the New York State Department of Agriculture and Markets volunteered to determine whether the material of the drying dishes had any influence on the quantity of sucrose inversion during the drying. The referee has received no report of the results of this work. He believes that if Miss Breen has been unable to finish the project, it should be restudied next year.

The association has previously recommended that the referee collect the methods relating to the analysis of fruit products under one heading so that reference to other sections need not be made. However, this does not seem advisable until a number of unfinished projects are completed and the correct answers to some open questions found. The referee believes that this task could best be undertaken when *Methods of Analysis* is revised¹.

No report on fruit acids was given by the associate referee.

REPORT ON ASH IN FRUIT PRODUCTS.

By DORIS H. TILDEN (U. S. Food, Drug and Insecticide Administration, San Francisco, Calif.), *Associate Referee*.

Proceeding along the lines of work indicated in the 1927 report on Ash in Fruit Products², methods for the determination of larger quantities

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 82.

² *This Journal*, 1928, 11: 445.

of manganese were studied, and a collaborative sample, covering determinations of the major bases in plant ashes, was sent out. Methods for the determination of iron and aluminum in plant ashes were not studied owing to lack of time.

For the manganese work a solution was prepared by taking a known volume of standard 0.1 *N* potassium permanganate, reducing it with sulfur dioxide, boiling off the excess, and making to volume. To ascertain the effects of large quantities of manganese on the calcium and magnesium determinations, when they occur in comparatively large quantities, a solution was prepared from reprecipitated C. P. calcium oxide and pure magnesium ribbon. Aliquots from the manganese solution and the calcium magnesium solution were combined in the required proportions at the time of analysis. The proposed scheme of analysis for these bases was followed. The manganese dioxide, precipitated in the acetic acid-bromine water-sodium acetate separation from calcium and magnesium, was determined both gravimetrically and colorimetrically. In some cases the colorimetric determination was made by dissolving the ignited manganomanganic oxide in sulfuric and nitric acid, or in hydrochloric acid, then adding sulfuric acid and evaporating the solution to fumes to remove all hydrochloric acid. In other experiments the precipitated manganese dioxide was dissolved directly from the filter and prepared for the colorimetric determination.

The results of experiments showing the recovery of large quantities of calcium and magnesium in the presence of considerable manganese are given in Table 1.

TABLE 1.

Recovery of large quantities of calcium and magnesium in the presence of large quantities of manganese.

MANGANESE (Mn_2O_4) RECOVERED (GRAVIMETRICALLY)		CALCIUM (CaO) RECOVERED	MAGNESIUM (MgO) RECOVERED
<i>mg.</i>		<i>mg.</i>	<i>mg.</i>
	48.4	139.4	
	50.9	139.7	
	51.5	139.9	
	49.0	140.0	163.91
	51.7		
Average	50.3	139.75	163.91
Theory	44.68	140.00	165.79

It is apparent that although the manganomanganic oxide recovered is higher than the theoretical amount, this excess is not due to occluded calcium or magnesium. It is also apparent that the proposed method yields satisfactory results for these elements when they occur in large quantities in the presence of large quantities of manganese.

To determine the effect of varying quantities of manganese on smaller quantities of calcium and magnesium, another sample solution was made up. The results of these determinations are given in Table 2.

TABLE 2.
Effect of varying quantities of manganomanganic oxide on the recovery of calcium and magnesium.

MANGANESE (Mn_2O_3)		CALCIUM (CaO)		MAGNESIUM ($MgCl$)	
THEORY	RECOVERED (GRAVIMETRIC)	THEORY	RECOVERED	THEORY	RECOVERED
mg.	mg.	mg.	mg.	mg.	mg.
(1) 44.68	49.7	30.0	30.2	41.44	41.49
(2) 22.34	24.4	70.0	68.6	82.89	84.67
(3) 22.34	25.7	15.0	15.3	20.72	22.51
(4) 11.17	12.2	35.0	35.1	41.44	42.86
(5) 11.17	12.2	7.5	8.0	10.36	11.51
(6) 10.13	11.6	100.0	100.0

From the figures given in Table 2 it appears that small quantities of calcium and magnesium are not affected by a comparatively large quantity of manganese, and that with care the manganese can be removed from the solution in one treatment. In the results recorded as Experiment No. 2, the low calcium and high magnesium are probably due to a faulty separation of these two elements.

The results obtained for manganese in the foregoing experiments are too high, although the probability of occluded calcium or magnesium is practically eliminated. In last year's work with the gravimetric method for manganese, smaller quantities of this element were used, and only a few results were obtained. The figures given in Table 3 show the percentage of error in this year's results. The only conclusion to be drawn from them is that possibly there is a general tendency for the error to decrease when smaller quantities of manganese are used, and that for large quantities the gravimetric method may be used only when it is desirable or necessary to sacrifice accuracy for speed. The colorimetric periodate method is applicable only to small quantities of manganese where the intensity of color produced is not great. Therefore, if the periodate method is used for large quantities, a series of dilutions is required.

TABLE 3.
Percentage of error occurring in the gravimetric determination of various quantities of manganese.

PRESENT	MANGANESE	RECOVERED	PERCENTAGE ERROR
mg.			
44.68	(Ave. 7)	50.14	+12.2
22.34	(Ave. 3)	24.87	+11.3
11.17	(Ave. 3)	12.20	+ 9.2
10.05	(Ave. 5)	12.19	+21.3
10.13	(Ave. 6)	11.50	+13.5
9.65	(Ave. 3)	10.77	+11.6
4.47		4.80	+ 7.3

To determine the effect on the colorimetric method for the determination of manganese of dissolving wet manganese dioxide and ignited manganomanganic oxide in hydrochloric-sulfuric acid, or sulfuric-nitric acid, the experiments recorded in Table 4 were performed.

TABLE 4.

Comparison of the gravimetric and colorimetric methods for the determination of manganese.

MANGANESE (Mn_2O_3)		PROCEDURE BEFORE USING COLORIMETRIC METHOD	MANGANESE (Mn_2O_3) RECOVERED (COLORIMETRIC METHOD)
PRESENT	RECOVERED (GRAVIMETRIC METHOD)		
mg.			mg.
9.66	11.3	Dissolved in H_2SO_4 and HNO_3	8.77
9.65	MnO_2 dissolved in H_2SO_4 and HNO_3	9.54
10.05	12.8	Dissolved in H_2SO_4 and HNO_3	9.57
10.05	12.7	Dissolved in HCl ; H_2SO_4 added and evaporated to fumes	8.99
10.05	14.1	Dissolved in H_2SO_4 and HNO_3	8.77
10.05	MnO_2 dissolved in H_2SO_4 and HNO_3	9.96
10.05	MnO_2 dissolved in HCl ; H_2SO_4 added and evaporated to fumes	9.54
10.05	10.36	Dissolved in H_2SO_4 and HNO_3	9.14
10.13	11.3	Dissolved in H_2SO_4 and HNO_3	10.4
10.13	10.8	Dissolved in H_2SO_4 and HNO_3	10.6
10.13	11.6	Dissolved in HCl ; H_2SO_4 added and evaporated to fumes	10.9
10.13	11.5	Dissolved in HCl ; H_2SO_4 added and evaporated to fumes	11.3
10.13	11.7	Dissolved in HCl ; H_2SO_4 added and evaporated to fumes	11.3

It is evident also from the results given in Table 4 that when extreme accuracy is necessary, the colorimetric method is more reliable than the gravimetric method. It may be used as a check on the gravimetric method, or may be applied directly to the manganese dioxide precipitated in the regular calcium-magnesium separation.

The solution to be used by the collaborators for the determination of major bases in plant ashes was prepared as follows:

mg. per 100 cc.

Calcium oxide.....	106.11	
Magnesium oxide.....	100.0	
Potassium oxide.....	126.3	(plus 6.2 mg. K_2O from the $KMnO_4$ used to supply the manganese)
Manganomanganic oxide.....	10.05	
Sodium dihydrogen phosphate.....	500.0	
Iron (wire).....	5.0	
Aluminum.....	4.17	

Determinations for potassium, manganese, calcium, and magnesium were made according to the proposed scheme of analysis.

PROPOSED METHODS FOR THE DETERMINATION OF POTASSIUM, MANGANESE, CALCIUM, AND MAGNESIUM IN PLANT ASHES.

POTASSIUM.

REAGENTS.

(a) *Ammonium chloride solution*.—Dissolve 100 grams of ammonium chloride in 500 cc. of water, add 5–10 grams of pulverized potassium platonic chloride, and shake at intervals for 6–8 hours. Allow mixture to settle overnight and filter. (The residue may be used for the preparation of a fresh supply.)

(b) *Platinum solution*.—For materials containing less than 15 per cent of potash, a platonic chloride solution containing 0.2 gram of metallic platinum (0.42 gram of H_2PtCl_6) in each 10 cc. is recommended.

(c) *90 per cent alcohol*.—Sp. gr. 0.8339 at $15.6^\circ/15.6^\circ\text{C}$.

PREPARATION OF SOLUTION.

(A) Dissolve the ash in hydrochloric acid. If it is desired to take an aliquot, filter into a volumetric flask, wash filter thoroughly, and make up to volume. Pipet an aliquot into a beaker, adjust to a volume of 50–75 cc., heat to boiling, and add a slight excess of strong ammonium hydroxide and then sufficient saturated ammonium oxalate solution to precipitate all the lime and aluminum present. Filter into a large platinum dish and wash filter thoroughly.

DETERMINATION.

Evaporate solution from (A) nearly to dryness, add 1 cc. of dilute sulfuric acid (1 + 1), evaporate to dryness, and ignite to whiteness. Maintain a full red heat until the residue is perfectly white. Dissolve the residue in hot water, using at least 20 cc. for each decigram of potassium oxide present; add a few drops of strong hydrochloric acid and then an excess of reagent (b). Evaporate on a water bath to a thick paste, avoiding exposure to ammonia. Treat the residue with 90 per cent alcohol. Filter, wash the precipitate thoroughly with 90 per cent alcohol, both by decantation and on the filter, continuing the washings after the filtrate is colorless, using about 200 cc. of wash solution. Then wash 5 or 6 times with 10 cc. portions of the ammonium chloride solution to remove impurities from the precipitate. Wash again with four or five 10 cc. portions of 90 per cent alcohol and dry the precipitate for 30 minutes at 100°C . Weigh, wash again with several 10 cc. portions of 90 per cent alcohol, dry, and reweigh until a constant weight of platonic chloride is obtained. Calculate to potassium oxide. The precipitate should be completely soluble in water.

MANGANESE, CALCIUM, AND MAGNESIUM.

REAGENTS.

(a) *Sodium hydroxide*.—10 per cent solution. (Do not use an old solution.)

(b) *Saturated solution of sodium oxalate*.

(c) *Sodium ammonium phosphate*.—10 per cent solution.

(d) *Dilute ammonium hydroxide*.—100 cc. of strong NH_4OH diluted to 1 liter.

(e) *Saturated solution of ammonium oxalate*.

PREPARATION OF SOLUTION.

(B) Dissolve the ash in concentrated hydrochloric acid, evaporate to dryness, and bake at 110°C . for 1 hour to dehydrate the silica. Dissolve the residue in dilute hydrochloric acid and filter into a volumetric flask. Wash filter thoroughly and make up to volume.

DETERMINATION.

MANGANESE.

To an aliquot of solution (B) add a few cc. of bromine water (until the solution is yellow) and boil until all the bromine is removed. (This oxidizes all the ferrous iron to the ferric state.) Make alkaline to methyl orange by adding reagent (a) drop by drop and stirring. Add acetic acid to make the solution acid (not over 1 cc. of concentrated acetic acid in excess). Boil and add a few drops of sodium dihydrogen phosphate solution to provide an excess of phosphoric acid. Filter if any precipitate of aluminum phosphate or ferrous phosphate occurs. Wash filter thoroughly and to the filtrate add 2 grams of sodium acetate and sufficient bromine water to color the solution distinctly yellow. Cover with a watch glass, bring to a boil, and boil briskly for a few minutes. Allow the precipitate to settle, add a little more bromine water, and boil again for a few minutes. Filter and wash thoroughly, reserving filtrate and washings for the calcium and magnesium determinations. Ignite the hydrated manganese dioxide gently at first, then strongly, to change it all to manganomanganic oxide. Weigh and report as manganomanganic oxide.

The manganese may also be determined colorimetrically by washing the manganese dioxide from the filter into a beaker with hot water and dissolving in 50 per cent sulfuric and 50 per cent nitric acid. The two acids used separately are necessary to secure complete solution. Add 5 cc. of concentrated sulfuric acid, boil to expel nitric oxide fumes, and proceed according to the official colorimetric periodate method (*Methods of Analysis*, p. 42, par. 8).

CALCIUM.

Adjust the volume of the filtrate from the manganese determination to 100–150 cc. Boil off any bromine remaining and precipitate calcium from the hot solution by adding reagent (b) drop by drop to slight excess. Continue boiling until the calcium oxalate begins to settle or digest for 15 minutes on the steam bath. Allow to settle until the solution is clear (usually not over 30 minutes is necessary). Filter off the calcium oxalate and wash thoroughly with hot water. Reserve the filtrate for the magnesium determination. Wash the precipitate carefully back into the original beaker, heat, and dissolve in as little concentrated hydrochloric acid as possible. Add ammonia drop by drop with continual stirring till the solution is slightly ammoniacal to methyl red. Then add acetic acid until slightly acid, and while still hot add a slight excess of ammonium oxalate (only a few drops will be necessary to provide an excess of oxalate). Digest on the steam bath for 1 hour and set aside until the precipitate settles clear, or overnight. Determine the calcium either gravimetrically or volumetrically by the usual methods. (For small quantities of calcium the gravimetric method is preferred.)

If magnesium is not to be determined, precipitate the calcium once from the boiling solution freed from iron, aluminum and manganese, with reagent (e); digest, and determine as described.

MAGNESIUM.

Concentrate the filtrate from the calcium precipitation to 50–75 cc. If a precipitate forms, which may occur when there is a large quantity of magnesium present, add a few cc. of concentrated hydrochloric to dissolve the precipitate, and continue evaporation to the desired volume. Heat to boiling, make slightly alkaline to methyl red with reagent (d) (added slowly), and add reagent (c) drop by drop in slight excess. Add one-third the volume of reagent (d) slowly with constant stirring and let stand 2 hours, or overnight. Filter, wash with reagent (d) until all chlorides have been removed, dry, burn at a moderate heat until the carbon has been mostly consumed, ignite intensely, and weigh as magnesium pyrophosphate. Report as percentage of magnesium oxide.

The only results obtained—those from the San Francisco Station—are given in Table 5.

TABLE 5.

Results on collaborative sample for the determination of calcium, magnesium, manganese, and potassium in plant ashes.

(Expressed as mg. per 100 cc.)

ANALYST	MANGANESE (Mn ₂ O ₄)		CALCIUM (CaO)	MAGNESIUM (MgO)	POTASSIUM (K ₂ O)	
	(Gravimetric)	(Colorimetric)			100 cc. aliquot	50 cc. aliquot
D. H. Tilden	12.8	9.57	105.5	99.90	128.0	64.0
	12.7	8.99	105.0	99.62	132.3
	14.1	8.77				
		8.77				
		9.96				
		9.54				
	11.0	106.2	98.40
G. T. Daughters	10.36	9.14	104.6	95.33	133.3	65.6
			107.0	95.51
				94.82		
Average	12.19	9.25	105.66	97.26	131.2	64.8
Theory	10.05		106.10	100.0	132.5	66.2

COMMENTS.

G. T. Daughters: Perhaps a word of explanation and comment would be helpful in interpreting my results. In the first place, I had difficulty in determining the end point when titrating to make just acid with acetic acid, previous to manganese separation, because of the discoloring of the indicator when near the end point.

You suggest that there be 1 cc. excess acetic acid, but do not advise whether the bromine be added while the solution is hot or cold. My results show that there is little or no difference in results caused by this change in temperature. Further, I find that samples having quite an excess of acid (3 cc.) seem to be no different from those run with a very slight excess (just acid to methyl orange).

My gravimetric results are higher than my colorimetric, and I wonder if this can not be explained by the fact that it is so difficult to reduce the manganese oxide to manganomanganic oxide. I found that by intense heating over a Fischer burner, in the presence of the charred filter paper, the results agreed more nearly with the colorimetric results. Perhaps this is caused by a mechanical loss of manganomanganic oxide from the burning, but I'm inclined to think that the carbon gave the desirable reducing atmosphere for the complete reduction of manganese.

From the results shown in Table 5, it will be noted that the proposed scheme of analysis for the major bases in plant ashes gives quite satisfactory results. A little difficulty was encountered in the first part of the manganese determination. After bromine water has been used to oxidize the iron, it is necessary to remove *all* bromine in order to get a sharp color change with the methyl orange indicator.

A solution similar to that used for the collaborative sample was prepared, but it was found to contain too much aluminum for the work in hand. Two determinations, however, were made in connection with the aluminum and iron separation. In one case the aluminum and iron phosphates were thrown down in the presence of macerated filter paper, filtered, and washed, and the manganese, calcium and magnesium were determined in the filtrate. In the second experiment, the iron and aluminum phosphates were precipitated as usual, dissolved, and reprecipitated. The manganese and magnesium were determined in the combined filtrates. The results are given in Table 6.

TABLE 6.

Recoveries of manganese, calcium and magnesium in the presence of large quantities of aluminum and iron phosphates.

(Results expressed as mg. per 100 cc.)

PRESENT	Mn ₂ O ₄ , CaO, AND MgO RECOVERED		
	AlPO ₄ and FePO ₄ precipitated in presence of paper pulp		AlPO ₄ and FePO ₄ reprecipitated
Mn ₂ O ₄ 9.65	{	colorimetric 8.77	9.54
CaO 99.60		gravimetric 11.30
MgO 100.0		97.5	97.0
Al ₂ O ₃ 18.85		99.3
Fe 10.0			

These results hold promise for future work with aluminum and iron, in the scheme of analysis under consideration.

SUMMARY.

(1) When the proposed scheme of analysis for plant ashes is followed, large quantities of manganese do not interfere with the calcium and magnesium determinations if these elements occur in comparatively large quantities.

(2) When large quantities of manganese, and small quantities of calcium and magnesium occur simultaneously in an ash, the manganese can be successfully removed by the acetic acid-bromine-sodium acetate treatment.

(3) Calcium and magnesium are not occluded by, nor do they occlude manganese, when this element occurs in varying quantities in an ash solution.

(4) Gravimetric manganese determinations show higher results than the theoretical, but results by the colorimetric method are quite satisfactory for small quantities.

(5) Results on a sample solution prepared for collaborative work on the major bases in plant ashes show the scheme of analysis proposed by the referee to be very satisfactory.

(6) The modification of the Lindo-Gladding method for potassium gives good results for large as well as small quantities of potassium.

(7) A comparatively large quantity of aluminum phosphate was successfully separated from manganese, calcium and magnesium by using the proposed method of analysis.

RECOMMENDATIONS¹.

It is recommended—

(1) That further collaborative work be undertaken on the determination of the major bases in plant ashes.

(2) That future work include combining the separation of iron and aluminum with their determination as phosphates.

REPORT ON CANNED FOODS.

By V. B. BONNEY (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

It is regretted that the opportunity to carry out the recommendation made last year with respect to canned foods did not present itself. It is felt that the study of methods for the detection of spoilage in canned foods should be undertaken, and it is recommended that this work be continued during the coming year.

In connection with the proposed study of the detection of field corn in canned corn, the work reported by Herd² shows the method of differentiation based on staining with iodine to give fairly concordant results in the hands of different analysts. These findings, it is believed, justify the recommendation for the adoption of the method as a tentative method¹. It is felt that the need for further study of the method is not urgent at this time, as this form of sophistication of canned corn is not being practiced to any extent. The method has been published³.

ADDRESS BY DR. WILEY.

LOUIS PASTEUR.

MR. PRESIDENT, MEMBERS OF THE ASSOCIATION,

LADIES AND GENTLEMEN:

The actual president of this association has one great advantage over me. He only has to give one speech, but evidently I have been selected to speak every year.

¹ For report of Subcommittee A and action of the association, see *This Journal*, 1929, 12: 82.

² *This Journal*, 1928, 11: 136.

³ *Ibid.*, 1929, 12: 39.

I greatly enjoyed the address of the president to which I have just listened; it was scholarly and to the point, well phrased, and read in a manner that everyone could understand. The points which he brought out are of great importance to agriculture and incidentally also to the health of humanity.

I have chosen today for the theme of my address another worker in agricultural chemistry, whose achievements in other lines have been so great that we have almost forgotten that he ought to be the honorary president of this association. I refer to Louis Pasteur.

Pasteur was born on the twenty-seventh of December, 1822, at a small town named Dole. His father was a small tanner. I mean that he had a small business which he prosecuted at this place. Previously he had been a soldier of the first emperor, and he was particularly active in the last days of the first empire, just prior to the surrender, in 1814, of Napoleon. He was in this campaign, the last one led by Napoleon, to save Paris from the enemy. You know the result of this campaign. Paris was taken by the combined allies and Napoleon was sent to the island of Elba in exile. For bravery on the field of battle, Mr. Pasteur, father of Louis Pasteur, was decorated in person by the Emperor Napoleon with the Cross of the Legion of Honor, which he valued as one of his most precious possessions.

Tanning at that time was not a chemical science; it was done by rule-of-thumb. The father of Pasteur, however, was a very good tanner and made excellent leather in a small way. He managed to live in a reasonably correct manner and was able to give his children such education as was provided. At that time when a child was promising or gave any exhibition of future greatness in any study, he was usually sent to Paris to complete his education. This was the fate of the young Louis Pasteur. He was sent to Paris to enter one of the great schools, which we would call colleges now in our country. There was the *École Normale*, for ordinary students, and the *École Polytechnique*, in which the young Frenchmen were trained for scientific and other purposes. Young Pasteur went to the *École Normale*, where he made very fine progress in chemistry; although he was not an honor student in the ordinary sense of that word, he commended himself to his teachers by his carefulness and by the accuracy of his work. He specialized in chemistry, and in his simple way and in the way in which chemistry was taught at that time became what was then known as a very good chemist.

His first published article, or one of the first, was on tartaric acid. He discovered that there are two kinds of tartaric acid, and he was the first one to call attention to that fact. One kind is the right-hand tartaric acid, and the other the left-hand tartaric acid. He noticed that when he tried to superimpose the right-hand tartaric acid on the left-hand tartaric acid, he got exactly what he would get if he tried to impose the

right hand upon the left hand. They did not agree exactly, as the right-hand glove is different from the left-hand glove. He found that there are two kinds of crystals, although they look just alike. One was the right-hand crystal, and the other the left-hand. This was a great discovery, which immediately led to quite a distinction on the part of this young Pasteur. It was the beginning of a series of discoveries in chemistry which were epoch-making in their character.

When he graduated from the *École Normale*, as we would from an ordinary college, he was assigned as teacher to some of the faculties of similar colleges in other parts of the country, and in all these cases he distinguished himself by the accuracy of his work, his enthusiasm, and the nature of his discoveries. It was the tendency of Louis Pasteur to keep after a thing until he unravelled any mystery or problem connected with it. I may cite his search for that kind of tartaric acid which was called then and is called now, racemic acid.

Most of the tartaric acid of commerce was either right-hand or left-hand, and he had received or got hold of some tartaric acid which had no rotation one way or the other (racemic acid). He was anxious to get larger quantities of this material. So he visited Italy, Spain, and many other countries where he thought he might find some of this neutral tartaric acid. He got very little of it. At that time they did not know how to make racemic or neutral tartaric acid by developing the right hand or the left hand or both together. They found that when they put one form in solution or the other form in solution, or put both together in solution, crystallization took place with the formation of neutral tartaric acid. In these efforts he spent almost a year trying to get some natural neutral tartaric, or racemic, acid.

The next thing Pasteur discovered was fermentation. At that time the general idea of fermentation was that it was autogenous, a development of life out of what had not been life before. Nearly everybody held to that theory—the theory of spontaneous generation. The next thing that Pasteur did was to show that that was an error, and he did it by sterilizing thoroughly articles which, left to themselves, would usually ferment, finding that as long as they were kept in this sterile condition no evidence of life existed. His theory that spontaneous generation was an error was not accepted by the chemists at that time. Everybody was against it. He spent years in experimentation, which was typical of his accuracy, before he got a hearing even before chemists in regard to this particular matter. The next great discovery, therefore, was that there is no such thing as spontaneous generation, that germs and eggs and progenitors cause the life and the decay of fermentable substances, and that they all have fathers and mothers and arise only as the ordinary life arises everywhere.

One of the men who fought that idea more desperately than any other was another great agricultural chemist, Liebig. Pasteur had corresponded with Liebig and knew that he held to the idea of spontaneous generation—that this life, whatever it was, that destroyed organic matter, was spontaneous and did not come from any life behind it. Finally he went to visit Liebig, and, while they had a pleasant conversation about other things, when they came to this subject, Liebig got up, excused himself, and said: "I cannot discuss this question with you". He never did accept Pasteur's theory that this spontaneous generation was just ordinary generation and was not spontaneous at all. There you see one great agricultural chemist arrayed against another on a fundamental principle, which can only be decided as Pasteur decided it, by actual experimentation. Finally, he brought everybody around to the belief of his own work by reason of the accuracy of the experimentation by which he illustrated it.

When he went to the college at Lille, as headmaster, he was appointed superintendent of manures, and in this position he made extensive examinations of all the kinds of manures which were used by farmers in that part of France. As you may know, Lille is in one of the most fertile agricultural regions of the French nation—right near Belgium. The land is naturally extremely fertile, and yet it is kept so by the manures that they use—the ordinary manures—not the kind we make now, but the kind the farmer makes himself. He analyzed these manures to show their quality.

His next great work in agriculture was the curing of sick wine or beer, mostly wine. He found that this trouble was due to the ferments that he had discovered, and he was the first one to pasteurize wine to destroy them. This was a great boon to the winemakers of France.

He undertook the investigation of the silkworm disease, since the growing of silkworms was a great agricultural industry in France. The silkworms dwindled and died, but no one knew the cause. The authorities called upon this agricultural chemist. He spent two or three years of his life in finding the real cause of the silkworm disease, and when he found it he proposed a remedy which was entirely satisfactory. He saved the silk industry of France by investigation and by prescribing for the sick silkworms. That was his next great contribution to French agriculture.

Pasteur invented the word "microbe" to distinguish these small animals or growing bodies, which nobody had identified and which could only be seen by the microscope. But "mikros" does not always mean little; it sometimes means short, and as the other part of the word was "bios", you wouldn't say "little lived", but that the individual was "short-lived". So the word microbe means short-lived. Strickland Gillian has described and written a poem about the microbe, which, if

you will pardon me, I want to recite to you, although it takes up considerable time. This is the poem of Strickland Gillian on the antiquity of the microbe—

“Adam had 'em.”

This poem is almost as famous as the one, “Off again, on again, gone again, Finnegan”.

The next thing was anthrax, which was destroying the sheep of France. Pasteur was called as an agricultural chemist to study this disease, and he did it in a most masterly way. It was many years, however, before he discovered its real character and before he discovered how the sheep and other farm animals that have it can be immunized against this disease. Of course he was familiar with Jenner's discovery of vaccination against smallpox and he noted the fact that it arose first on the cow. That led him to believe that the proper way to study these diseases was on farm animals. In that way he laid the foundation of the great studies on immunization which have proved so valuable to the human family. Jenner never had any idea why vaccination cured smallpox or prevented it. It was Pasteur who discovered that idea and the real reason of its existence. He reasoned that the way to immunize an animal against anthrax is to attenuate the disease which kills animals to such an extent that it can be injected into other animals. In this way the slight attack of the disease that follows immunizes the animal against a severe attack.

That is the foundation of the great work that now is universally accredited to Pasteur as its author. He had great trouble in attenuating the disease so that it would not kill the animal into which it was injected, but he finally discovered the method which would do it. It is too long for me to go into the discussion of it. It is now well understood that you can produce a mild form of smallpox, called variola, by the serum that is used against smallpox. Then, if you do have smallpox, you have only a modified form of it. In this way Pasteur finally vaccinated animals against anthrax. He had great difficulty to get anybody to believe it, but this is the way he proceeded. He said, “Now, I will vaccinate, say, 25 sheep against anthrax, and then I will put them, together with 25 other sheep that are not vaccinated, in with an animal that has already developed this disease and is capable of communicating it to all 50 of these sheep, and I will guarantee that not one of these that I immunize will die and that those not immunized will die”. By carrying on experiments of this kind he convinced the farmers first of all that what he said was true, and finally he convinced the scientific men.

Just last week a marble shaft, erected on the lake shore in Chicago, was dedicated to the honor of Pasteur. The first person that ever

crossed the Atlantic from this country to take the treatment for rabies from Pasteur himself was present—a man by the name of Lane. After being bitten by a mad dog he sailed for Paris and was immunized by Pasteur. That was the only place you could be immunized at that time. He never had rabies. That was a most appropriate thing—to have the man who was the first American ever immunized against rabies present when that shaft was unveiled. It is a wonderful tribute to this great agricultural chemist, who was a great chemist in other directions, as I have already shown.

Then came the immunization against many other diseases, but unfortunately Pasteur never won over all diseases. As a result he lost two children, one at four years of age and one at twelve years of age, the latter by typhoid fever. At the present time we know how to control this disease so well that there is no danger whatever if we take the immunization treatment that we will ever die of it. His love for his family was one of the characteristics of this man, and his heart was broken by the death of his two little daughters.

What is the reason that you can get such perfect protection against rabies and you don't get this same protection against diphtheria, which is treated in the same way? I'll tell you. After you are bitten by a mad dog, it is rarely that you become mad within two or three weeks. Rabies is a long while in developing. When a child has diphtheria, a doctor is usually not called until two or three days afterward, and it is such a dreadful disease that it is then too late to immunize. If you want to immunize against diphtheria, we have a certain way of doing it. Most children have in their own bodies the immunizing agents against diphtheria so they will not have it under any circumstances, but some children do not have these agents. However, by administering toxin and antitoxin to children in general, we can distinguish those that are not immune to diphtheria, and then by proper dose of the toxin and antitoxin every child can be made immune. But parents don't want to do this, or they don't think of it. They wait until the children are seized with diphtheria. They don't know what it is, and when they do call a doctor it is usually too late. On the other hand, sometimes rabies does not develop for a year, and there is ample time to immunize.

Pasteur also took dogs and put them in a pen with a rabid dog; then he took the same number that he had immunized against rabies, and in every instance every dog that was not immunized died of rabies and no dog immunized died of the disease. And yet he was afraid to try it on a human being. He was very careful indeed before he injected anything into a human being. Finally a case came to him from Strasburg, where he once lived as a teacher, a man badly bitten by a mad dog. He called in his colleagues in the scientific world in Paris and laid the matter before them. He said, "See, here is what I have done with dogs. Now, shall

I try it on this man"? They all agreed and said, "Yes". And although the patient was badly bitten and scratched about the face, he never had rabies, and no one else ever has who has been immunized in the proper way after being bitten. He found immediately it was not the saliva that did the work, although it may do it, but when he went to immunize people he went deeper. He examined the brains of animals and men who died from rabies, and he found that at the base of the brain was the affected tissue; so when he made his immunizing agents, he took out that part of the brain and used it for the attenuation of his serum. He went right to the place where the disease is most violent but which develops the antibody that protects you. In numerous places in this country we have institutes where immunization can be given. So it is not necessary to go to Paris when a person is bitten by a mad dog.

Pasteur was not only a great agricultural chemist, doing more for the agriculture of France than any other one man ever has done, but he was a great humanitarian. He loved the human family. He wanted to do something to protect it against disease. He discovered the nature of all contagious diseases, and that they are all due to microbes. Before his death he developed a number of attenuated serums which were perfectly valid and successful in protecting his fellowmen from these particular diseases. That is the reason he is so loved and honored by all the country.

Long before his father died, the Republic of France recognized the great value and service of Louis Pasteur and made him also a chevalier of the Legion of Honor. Then his father, who had received his cross direct from the Emperor Napoleon, and his son, who had received his cross from the French Government, were both members of the Legion of Honor and both valued very highly that acknowledgment of their work. So Pasteur was the great humanitarian, the great agricultural chemist, the great lover of his race, of his time, and of his country. His devotion to France was something most remarkable; he loved France not only because he was a citizen of it, but because it was France. He received an honorary degree from the University of Bonn for his career in his chosen profession. In the war of 1870, when the German army overran France and captured Paris, he was so hurt that immediately after the war was over he sent back his diploma, refusing to keep it longer, as an index of what he regarded as a great indignity to France, the separation of Alsace-Lorraine from France and its addition to the German Empire. He never forgave the Germans for that. He never lived to see the reparation, but his successors have lived to see the reparation of that great insult to the integrity and dignity of France. And so for this love of his country and of his fellowmen, and for his great accomplishments in every field of chemistry he was made a member of the Academy of Medicine—although he was not a graduate of any

medical school—of the Academy of Science—of which he was well prepared to be a member—and of the French Institute. He became one of the Forty Immortals. That is the highest distinction a Frenchman can receive in his own country.

He died on the twenty-eighth of September, 1895, and was buried in the yard of the Pasteur Institute in Paris. When I went to Paris in the spring of 1896, I was told that I ought to visit at once the church of Notre Dame, the great cathedral in Paris, because there I would see still stored in that great church the testimonials of all the world sent to Paris at the time of the death of Pasteur. When I came into that gigantic dome of the great cathedral, I saw everywhere, in every recess, hundreds of wreaths of immortelles that had been sent to Paris several months before at the funeral of Pasteur and were still stored in that great church. That was a most imposing evidence of the admiration of the whole world for this great man and humanitarian—agricultural chemist, founder of immunization, patriot—Louis Pasteur.

SECOND DAY. TUESDAY—AFTERNOON SESSION.

REPORT ON CEREAL PRODUCTS

By J. A. LeCLERC (Bureau of Chemistry and Soils, Washington, D. C.),
General Referee.

It is 27 years since the first Associate Referee on Cereal Products was appointed. A. M. McGill of Canada served in that capacity for six years, 1901–1907. He was followed by E. F. Ladd, 1907–1909; W. L. Allen, 1909–1910; H. L. White, 1910–1913; B. R. Jacobs, 1913–1914; L. A. Fitz, 1914–1915; J. A. LeClerc, 1915–1919; C. H. Bailey, 1919–1922; C. E. Mangel, 1922–1923; Raymond Hertwig, 1923–1925; and F. C. Blanck, 1925–1927.

During those 27 years the association has adopted as official the methods for the determination of the following constituents: In flour—water, ash, crude fat, crude fiber, protein, water-soluble nitrogen precipitable by 40 per cent alcohol, lipoids, lipid phosphorus pentoxide, and fat (by acid hydrolysis); in macaroni—ash, protein, chlorides in ash, organic and ammonium nitrogen, added color; in bread and baked cereals—ash and protein. The methods adopted as official, first action, are sampling and hydrogen-ion concentration of flour, lipid and lipid phosphorus pentoxide, and fat by acid hydrolysis in macaroni and crude fat in baked products. Tentative methods have been adopted for the following determinations: In flour—acidity in water extract, sucrose, reducing sugars, alcohol-soluble protein, 5 per cent potassium sulfate soluble protein, globulin, albumin, amino nitrogen, glutenin, gluten, cold water extract, chlorine, NO_2 , gasoline color value, starch, F. A. C. unsaponifiable matter in fat and the Kerr-Sorber unsaponifiable matter in fat; in bread and baked products—moisture and preparation of sample; in alimentary pastes—moisture, preparation of sample, water-soluble nitrogen precipitable by 40 per cent alcohol, detection of egg and egg yolk, egg solids, F. A. C. and Kerr-Sorber unsaponifiable matter in fat.

Thus far the association has adopted no official nor even tentative method for the following: In flour—gliadin, detection of benzoyl peroxide, detection of rye in flour, the baking value of flour, ash constituents; in alimentary paste—crude fat, crude fiber, acidity, starch; in baked products—crude fiber, acidity, sucrose, dextrose, cold water extraction, chlorides in ash, lipoids, lipid phosphorus pentoxide, detection of egg (white or yolk), starch, fat by acid hydrolysis, unsaponifiable matter in fat.

Collaborative work has been conducted only on such products as flour, alimentary paste, and bread. No methods are recognized as official or

even tentative for the cereals themselves, nor for cakes, pies, pretzels, doughnuts, and other baked products, all of which are becoming of greater and greater importance. Thus, while much has been accomplished, much remains to be done, as the field appears to be growing faster than are the activities of the association.

That cereal chemistry has "arrived" is manifest not only by the tremendous scope of the work and by the large amount of energy now being devoted to this branch of A. O. A. C. activities, but by the further fact that within quite recent years there has been formed a vigorous and thriving organization of cereal chemists, over 350 strong, with its own publication, a bimonthly journal ranking second to none in the whole field of cereal chemistry.

Too much praise cannot be bestowed upon the past referees, associate referees and collaborators for their self-sacrificing, loyal and efficient services, which have produced results of such far-reaching importance. The present associate referees and collaborators deserve the same credit. Their self-sacrifice, their hope of benefitting cereal chemists of the future just as they themselves have been benefitted by their predecessors, and their idealism are making progress possible.

The preceding referee laid out an ambitious program for this year's work, covering some 18 subjects in all. While it has been found impossible to cover every field, all the work planned by the past referee will eventually come into fruition.

SAMPLING OF FLOUR.

Associate Referee H. Runkel reports that inasmuch as no adverse criticism has been noted in respect to the procedure employed for the sampling of flour¹, he recommends that the tentative method be adopted as official, first action.

ASH IN FLOUR.

No collaborative work was done on the subject of ash during the past year. Numerous suggestions have been made to shorten the time of ashing, and these should of course be seriously studied by next year's referee.

GASOLINE COLOR VALUE.

As in the case of ash, Referee Coleman found it impossible to report on the gasoline color value of flour.

GLUTENIN IN FLOUR.

M. J. Blish, referee on glutenin in flour, obtained results from 11 collaborators on his method, based upon the use of barium hydroxide and

¹ *This Journal*, 1928, 9: 39.

methanol. The results varied from 3.98 to 4.78 per cent, with an average of 4.36 per cent glutenin. The average probable error of each individual analyst was calculated to be ± 0.154 .

Eight of the 11 collaborators secured results which the associate referee regards as well within reasonable expectations. This close agreement is doubtless due to the fact that all the collaborators used the same reagents.

His recommendation is that inasmuch as this method is the simplest of all known procedures and besides gives results of accuracy and reliability sufficient for present requirements and purposes, the barium hydroxide method for the estimation of glutenin in flour, as specified in his report, be made tentative. This method has been published¹.

HYDROGEN-ION CONCENTRATION OF FLOUR.

The associate referee on this subject, C. H. Bailey, reported that "further research is necessary before any more progress can be made", and "that efforts would be made to promote such research at the earliest possible opportunity".

DIASTATIC VALUE OF FLOUR.

E. L. Tague reported that for the past two years he had been (1) searching for a uniform substrate, one that would be more satisfactory than are the starches, and (2) investigating new methods for determining sugar.

As a substrate, glycogen appeared to be most promising. The commercial product, however, contained varying amounts of reducing sugars as well as an enzyme that broke down the glycogen itself into its component reducing sugars. This made it necessary to investigate methods of preparing a glycogen free from those defects, which problem remains unsolved. The associate referee has found no better method for determining reducing sugars than that of Munson and Walker.

STARCH IN FLOUR.

The associate referee on starch in flour, L. H. Bailey, was directed to compare the so-called Rask method², which was adopted as tentative, with the Hartmann-Hillig modification of the diastase method³. Only two chemists sent in reports. These results showed that the tentative method gives slightly higher results. It is recommended that further work be conducted on this subject.

CHLORINE IN BLEACHED FLOUR.

Associate Referee G. C. Spencer has once more conducted collaborative work on the Seidenberg modification⁴ of the method to determine

¹ *This Journal*, 1929, 12: 39.

² *Ibid.*, 1928, 11: 37.

³ *Ibid.*, 1926, 9: 483.

⁴ *Ibid.*, 1928, 11: 132.

chlorine in bleached flour. Based upon the results received from seven collaborators, he recommends that, although this method is probably the best yet devised for this purpose, further work be done on it.

SAMPLING OF BREAD AND DETERMINATION OF MOISTURE.

This work has been carried on by Associate Referee L. H. Bailey. A loaf of pan bread was cut into four as nearly equal parts as possible. The moisture content in each quarter was found to be as follows: 34.49, 35.06, 35.14, and 35.78 per cent; average, 35.12 per cent.

These results show a variation of 1.3 per cent in the moisture content of different portions of the bread, the maximum deviation from the average being 0.63 per cent. It is believed that these differences would be greater in the case of the Vienna or French type of bread.

The associate referee recommends that the entire loaf constitute the sample for analysis and that the tentative method for the total solids (or its complement moisture) in an entire loaf¹ be made official (first action).

LIPIDS AND FAT IN BREAD.

A comparative study was made of the acid hydrolysis method for fat and the tentative method for fat² in baked products. This work was under the direction of Associate Referee Samuel Alfend. Six collaborators assisted. The results for fat by the acid hydrolysis method are satisfactory, and it is recommended that this method be adopted as tentative. The results for lipoids showed considerable irregularity, varying from 3.62 to 5.39 per cent. It is recommended that further work with this method include the determination of lipid phosphorus pentoxide.

MILK SOLIDS IN BREAD.

Associate Referee L. H. Bailey conducted a rather extensive investigation of the methods for the determination of milk solids in milk bread, based upon the lactose and casein content. The results, however, were so unsatisfactory and the methods used were so unreliable that it is recommended that collaborative work on this subject be temporarily dropped. This investigation should be assigned to some investigator for special work.

EXPERIMENTAL BAKING TEST.

M. J. Blish acted as Associate Referee on Standardization of the Experimental Baking Test for this association and as chairman on the same subject for the American Association of Cereal Chemists. An extensive report was published³. A definite, fixed baking procedure was formulated.

¹ *This Journal*, 1926, 9: 42.

² *Methods of Analysis*, A. O. A. C., 1925, 231.

³ *Cereal Chem.*, 1928, 5: 158.

This procedure meets the following major requirements to a greater degree than any other known method: (1) Strict adherence to the principle of fixed specifications whereby the material to be tested is the only variable; (2) usefulness in permitting the ascertainment of differences among flour characteristics, and the translation of these differences into terms of commercial utility; and (3) maximum simplicity and conservation of time, material, labor, and equipment. The method proposed by the associate referee is the result of over two years' investigation by a committee of the A. A. C. C. and has been officially approved and adopted as a tentative standard method by that organization. The associate referee recommends therefore that this same method be adopted as tentative by this association. The method has been published¹.

UNSAAPONIFIABLE MATTER IN FLOUR AND ALIMENTARY PASTES.

Associate Referee Samuel Alfend assisted by four collaborators gave considerable time and attention to the determination of the unsaponifiable matter in the extracted lipoids by the modified F. A. C. method². The results were not satisfactory. Inasmuch as this is the best method available, the associate referee recommends that no further collaborative work be done on it for the present, and that it be adopted as official.

WATER-SOLUBLE PROTEIN PRECIPITABLE BY 40 PER CENT ALCOHOL IN ALIMENTARY PASTE.

Based upon results from six collaborators, Associate Referee Alfend recommends that the official method for this determination³ be dropped and that the modified method proposed by him be adopted as tentative. This is really the same method with certain slight modifications of procedure.

MOISTURE IN ALIMENTARY PASTES.

Referee S. C. Rowe with the aid of five collaborators made a study of the official vacuum method⁴ and of the air-oven method⁵ for the determination of moisture in alimentary pastes and recommends that both of these methods, which are official for flour, be likewise adopted as official (first action) for alimentary pastes.

RECOMMENDATIONS OF THE REFEREE⁶.

Flour.

(1) That the associate referee continue studies on rapid methods of ashing flour (bread and alimentary pastes), giving special attention to the use of a glycerol-alcohol mixture.

¹ *This Journal*, 1929, 12: 41.

² *Ibid.*, 1928, 9: 45.

³ *Ibid.*, 429.

⁴ *Ibid.*, 39.

⁵ *Ibid.*, 1928, 11: 37.

⁶ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 84.

(2) That the associate referee study the nature of the losses occurring when ash is fused.

(3) That the tentative method for sampling flour¹ be adopted as official (first action).

(4) That the F. A. C. method for the determination of unsaponifiable matter in fats and oils² as modified and adopted as tentative for flour³ be retained as tentative.

(5) That the method for the determination of glutenin specified by the associate referee be adopted as tentative.

(6) That the associate referee continue research on the official (first action) method for the determination of hydrogen-ion concentration of flour, this to include a comparison of the use of the quinhydrone and antimony electrodes.

(7) That the study of the methods for the determination and valuation of the diastatic power in flour be continued.

(8) That further study be conducted on the Seidenberg method and modifications thereof for the determination of chlorine in bleached flour.

(9) That further study be made of the (Rask) tentative method⁴ of determining starch in flour (bread and alimentary pastes), and that this method be compared with the diastase method as modified by Hartmann and Hillig⁵.

(10) That the factor for the converting of percentage of nitrogen into protein in wheat (viz., 5.83) as suggested by Jones⁶, be adopted only in so far as it relates to scientific work; that for all commercial transactions and regulatory work the old factor 5.7 be retained.

(11) That the official method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol⁷ be dropped and that the method as modified by the associate referee be made tentative. This method has been published⁸.

(12) That the associate referee on bleaching of flour be directed to study methods to detect the use of benzoyl peroxide.

(13) That consideration be given to the study of certain foreign methods of analysis, especially those which are used by foreign governments in testing flour imported from this country.

Alimentary Pastes.

(1) That the tentative method for collecting and preparation of sample of alimentary paste for analysis⁹ be further studied with the view to making it official.

¹ *This Journal*, 1926, 9: 39.

² *Ibid.*, 41.

³ *Ibid.*, 1928, 11: 37.

⁴ *Ibid.*, 1927, 10: 108.

⁵ *Ibid.*, 1926, 9: 482.

⁶ *Cereal Chem.*, 1926, 3: 197.

⁷ *This Journal*, 1926, 9: 40.

⁸ *Ibid.*, 1929, 12: 40.

⁹ *Ibid.*, 1926, 9: 43.

(2) That the methods now official for the determination of moisture in flour¹ be adopted as official (first action) for alimentary paste.

(3) That further collaborative work be done with the tentative F. A. C. method for the determination of the unsaponifiable matter in the fats of alimentary paste (and baked cereal products).

(4) That the tentative method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol² be dropped and that the modification as proposed by the associate referee be made official (first action). This method has been published³.

(5) That the official method for the determination of fiber in flour⁴ be made official (first action) for alimentary paste.

Baked Cereal Products.

(1) That the referee make further studies of the tentative method for sampling of bread⁵, especial attention being paid to different types of bread.

(2) That the tentative method for the determination of total solids in an entire loaf of bread⁶ be made official (first action).

(3) That the official method for the determination of fat⁶ (by acid hydrolysis) in flour be adopted as tentative for baked products and that a comparative study be made of this method with the present tentative method⁶ for fat in baked cereal products.

(4) That further study be made of the methods to determine lipoids in baked products.

(5) That the standard experimental baking test proposed by the associate referee be adopted as tentative and subjected to collaborative study.

(6) That an associate referee be appointed to carry on research looking toward the development of methods for the determination of milk solids in bread.

(7) That consideration be given to the development of methods for the estimation of rye flour in rye bread.

(8) That the official method for the determination of chlorides in the ash of alimentary paste⁷ be made official (first reading) for baked cereal products.

(9) That the official methods for moisture determination in flour⁸ be made official (first action) for the air-dried baked cereal products.

(10) That the official method for the determination of fiber in flour⁹ be made official (first action) for the air-dried baked cereal products.

¹ *This Journal*, 1926, 9: 34, 40.

² *Ibid.*, 39, 40.

³ *Ibid.*, 1929, 12: 40.

⁴ *Methods of Analysis*, A. O. A. C., 1925, 225.

⁵ *This Journal*, 1926, 9: 42.

⁶ *Methods of Analysis*, A. O. A. C., 1925, 231.

⁷ *Ibid.*, 232; *This Journal*, 1927, 10: 34.

⁸ *This Journal*, 1926, 9: 39-40.

⁹ *Methods of Analysis*, A. O. A. C., 1925, 225.

(11) That the official method for the determination of organic and ammonium nitrogen in alimentary paste¹ be made official (first action) for the air-dried baked cereal products.

(12) That consideration be given to the study of baked products other than bread.

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No report on sampling of flour was given by the associate referee.

No report on ash in flour and gasoline color value was given by the associate referee.

REPORT ON GLUTENIN IN FLOUR.

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Nebr.),
Associate Referee.

The work of the past year was confined exclusively to the recommendation of the previous report¹. Collaborative studies were carried on involving the use of the barium hydroxide method² only, with special precautions to insure that all collaborators used the same reagents and the same procedure in minute detail.

This report is based upon the results of eleven collaborators. Each collaborator was furnished, in a sealed container, a representative sample of a "95 per cent patent" baker's flour of average protein content for a flour of that type. Each lot of flour was accompanied by a suitable portion of synthetic methanol, as well as a small sample of barium hydroxide taken from a fresh lot. The recommended procedure has been published³.

The collaborative results are indicated in Table 1.

TABLE 1.
Collaborative analytical data.

COLLABORATOR	TOTAL PROTEIN per cent	GLUTENIN* per cent	PROTEIN- GLUTENIN RATIO
D. A. Coleman	11.42	4.56	2.50
H. C. Fellows	11.36	4.22	2.69
F. A. Collatz	11.40	4.30	2.65
C. E. Mangels	11.35	4.25	2.67
L. H. Bailey	11.23	4.35	2.58
J. T. Flohil	11.40	4.63	2.46
W. L. Heald	11.33	3.98	2.85
B. L. Herrington	11.41	4.57	2.50
G. S. Taylor	Not reported	4.10	
R. C. Sherwood	11.46	4.78	2.40
H. Platenius	11.29	4.24	2.66
Average	11.37	4.36	2.60
Maximum	11.46	4.78	2.85
Minimum	11.23	3.98	2.40
Range	0.23	0.80	0.45
Standard deviation	± 0.069	± 0.228	
Probable error of individual analyst	± 0.046	± 0.154	

* Average of 3 determinations.

The data presented in Table 1 show a closer concordance of results than was the case in a similar series accounted for in last year's report, and involving the same number of collaborators. Last year's sample had a protein content of 11.41 per cent and contained 4.31 per cent glutenin. The present sample contained 11.37 per cent total protein and 4.36 per cent glutenin, being for all practical purposes identical with the sample of last year. The maximum range of variation in glutenin

¹ *This Journal*, 1928, 11: 475.

² *Cereal Chem.*, 1927, 4: 129.

³ *This Journal*, 1920, 12: 39.

for last year was 1.24, while for this year it was 0.80. In the present series 8 of the 11 collaborators, as against 7 out of 11 for last year, secured results which the associate referee regards as well within reasonable expectations for a method which essentially undertakes a sharp and precise separation of two proteins which are similar in many of their properties. The smaller range of maximum variation in this year's results is doubtless due to the fact that all collaborators used the same reagents, as well as to a closer specification of the details of procedure.

The table includes the results of a statistical analysis of the data, employing formulas which are widely used by biometricians. Although the number of collaborators is smaller than desirable for statistical studies of this type, the indication is that when a sample of average baker's flour is submitted to a qualified chemist for the estimation of glutenin by the barium hydroxide method, the result will be reliable within plus or minus 0.154, in terms of per cent of glutenin in the flour.

The associate referee feels that present circumstances do not warrant any further attempts to improve the accuracy or reliability with which glutenin in wheat flour may be estimated by the barium hydroxide method. The ascertainment of the percentage of glutenin in flour is rarely, if ever, resorted to in industrial practice, for the reason that its practical significance and application have never been definitely established. Since, furthermore,—as appears from the work of numerous investigators—the ratio of total protein to glutenin is fairly constant, even among flours of widely different type, grade, origin and protein content, a knowledge of the total protein content of the flour may serve for most purposes as a basis upon which glutenin may be reliably calculated.

Summarizing the entire glutenin situation, it may be stated that although certain recent investigations are beginning to cast some doubts upon the validity of the present conception of the identity and individuality of glutenin, Osborne's¹ characterization is still generally adhered to. Based upon this characterization, the barium hydroxide method gives results of accuracy and reliability sufficient for present requirements and purposes, and it is the simplest of all known procedures. Accordingly, the barium hydroxide method for the estimation of glutenin in wheat flour, as published previously, is recommended as a tentative method².

No report on hydrogen-ion concentration of flour was given by the associate referee.

¹ *The Proteins of the Wheat Kernel*. Carnegie Institution of Washington, 1907.

² For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 85.

REPORT ON DIASTATIC VALUE OF FLOURS.

By E. L. TAGUE (State Agricultural College, Manhattan, Kan.),
Associate Referee.

For the past two years the associate referee has been working on a method for determining the diastatic activity of flours. The first year was spent in (1) searching for a uniform substrate, (2) investigating new methods for determining reducing sugars.

(1) Of the several substances investigated, glycogen was found to be the most promising and to possess many advantages as a substrate. Since it is soluble in water (at least to a colloidal solution) its concentration can be varied, and the action of the diastase is much more rapid. In addition, the glycogen does not vary so much in physical qualities as do the starches from different flours.

The disadvantage of using the glycogen on the market is due to the varying amounts of reducing sugars it contains and also to an enzyme which breaks down the glycogen itself into its component reducing sugars. These defects made it necessary to investigate the preparation of glycogen. Accordingly, the past year was spent in studying methods suitable for this purpose. It was found that if the crude material (liver) is worked up immediately after its removal from the animal body, the reducing sugars are absent. The enzyme, however, cannot be removed without deleterious changes in the glycogen. For instance, the glycogen is rendered less soluble in water and is broken down into reducing sugars and dextrin-like substances. As a result, the utility of glycogen as a substrate awaits a new method of preparation.

(2) No better method for determining reducing sugars than that of Munson and Walker¹ can be recommended².

STARCH IN FLOUR.

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The associate referee was directed to make further collaborative study of the tentative method for the determination of starch³ and to study the modification of the diastase method for starch suggested by Hartmann and Hillig⁴.

Unfortunately, several persons who had signified a willingness to collaborate on the tentative method of determining starch did not find it possible to do so. The few results that were obtained showed fair agree-

¹ *Methods of Analysis*, A. O. A. C., 1925, 190.

² For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 85.

³ *This Journal*, 1928, 11: 37.

⁴ *Ibid.*, 1926, 9: 482.

ment, but it is thought that more results should be obtained before it is recommended that the method be made official. The associate referee has had considerable experience with this method in his own laboratory and is well pleased with it.

The collaborative results follow.

COLLABORATOR	PER CENT OF STARCH
A. G. Buell, Food, Drug and Insecticide Administration, Chicago, Ill.	71.5 71.6
L. H. Bailey	73.52 73.54

It is recommended that further collaborative work be done on this tentative method with a view to having it adopted as an official method.

The method proposed by Hartmann and Hillig was also studied by the associate referee. The flour was washed with ether, 70 per cent alcohol, and water, and was then digested overnight with a solution of pepsin. Malt infusion was added, and the process was completed as in the regular diastase method.

The digestive action of the pepsin on the proteins renders them soluble and so liberates the starch that it is acted on more readily by the diastase. The percentage of starch obtained by this method on the sample of flour used in the tentative method was as follows: 72.98 per cent, 73.05 per cent, and 72.68 per cent. These results are slightly lower than those obtained by the tentative method, and no advantage is seen over that method. This method is *not* recommended for adoption¹.

REPORT ON FLOUR-BLEACHING CHEMICALS.

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

The work of the past year was confined to a collaborative study of the Seidenberg method² for the estimation of added chlorine in bleached flour.

After this method had been carefully reviewed by the associate referee, the following instructions were prepared for the guidance of the collaborators:

Please follow Seidenberg's revised directions * * *. I recommend, however, that the contents of the platinum dish be dried in an air oven before starting to burn off the organic matter; see lines 1 to 7 on page 135. After driving off the volatile matter with a low flame, it is better to complete the ignition in a muffle furnace below red heat if a muffle is available in your laboratory, otherwise the final filtrate is apt to be slightly

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 85.

² *This Journal*, 1928, 11: 132.

colored. If you are not entirely familiar with the titration of silver under these conditions, you had better practise with the two 0.005 *N* solutions a number of times in order to accustom your eye to the end point.

Collaboration was solicited from fifteen chemists who had signified a willingness to assist in cereal investigations. Ten of these asked for flour samples to work with, but only seven sent in a final report.

The following table of results is submitted:

Chlorine in bleached flour.
(Results expressed in parts per million.)

COLLABORATOR	1	2	3	4	5	MAX.	MIN.	AVE.
L. H. Bailey.....	46.1	44.2				46.1	44.2	45.1
F. B. Carpenter, Jr.....	48.0	54.0	56.0			56.0	48.0	52.0
A. J. Christie, Jr.....	38.0	40.0				40.0	38.0	39.0
Emily Grewe.....	46.1							46.1
O. F. Krumboltz.....	51.0	48.0	40.0	45.0	46.0	41.0	40.0	46.0
W. C. Luckow.....	27.8							27.8
F. T. Shutt.....	32.0	31.0	30.0	45.0		45.0	30.0	34.0
G. C. Spencer.....	56.4	59.3	52.3	46.5	56.1	59.3	46.5	54.1

The tabulated results seem to indicate that two general conclusions may safely be drawn concerning this method: (1) That the method is probably the best that has thus far been proposed for added chlorine in flour; (2) that the directions given by Seidenberg must be rigorously followed if concordant results are to be expected.

RECOMMENDATIONS¹.

It is recommended—

That further work be done on the Seidenberg method for the determination of chlorine in flour.

REPORT ON METHODS FOR BREAD ANALYSIS.

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.),
Associate Referee.

Preparation of sample, determination of moisture in bread, and milk solids in milk bread were the subjects assigned.

SAMPLING AND DETERMINATION OF MOISTURE.

For the first two studies the associate referee baked two loaves of bread under as nearly the same conditions as possible. One loaf was sliced in thin slices and dried in the open air until air-dry; then the slices were ground, and the moisture was determined in the air-dry product.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 85.

From this result the total solids of the loaf were calculated as 64.6 per cent.

The other loaf was quartered, and each quarter was sliced and air-dried and then ground separately. Moisture was determined on the air-dry portions, and from these results the total solids of each quarter were calculated. The results were as follows: 65.51, 64.94, 64.86, and 64.22 per cent. The average for the loaf was 64.88 per cent. The maximum variation among the quarters was 1.29 per cent, and the maximum variation from the mean was 0.63 per cent. This experiment shows that variations in total solids content exist in different portions of a loaf of bread. This is to be expected, especially in a loaf of irregular shape where the proportion of crumb and crust varies in different parts of the loaf. Since such variations are shown to exist, the only way to obtain the correct result is to use the entire loaf for the sample. Therefore it is recommended that an entire loaf of bread should constitute the sample for analysis.

Moisture in bread or its complement, total solids, is determined very satisfactorily by the tentative method¹, but this method is not susceptible to collaborative study for the reason that an entire loaf is taken as the sample and any other loaf would be a different sample. It is recommended that this tentative method be made official (first action)².

MILK SOLIDS IN MILK BREAD.

For the third study the associate referee made an extensive investigation of methods for the determination of milk solids in milk bread. Various methods based on the lactose content of the bread were tried, but none of them proved to be satisfactory. Methods that were based on the casein content were also tried, but they likewise were unsatisfactory. Since these were the only factors that gave promise of differentiating milk bread from ordinary bread, and since no reliable method could be found that would indicate quantitatively the amount of milk present in breads of known milk content, the problem was abandoned.

REPORT ON LIPOIDS AND FAT IN BREAD.

By SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.), *Associate Referee*.

A sample of air-dried white bread, ground to pass a 20-mesh screen, was sent out for collaborative determination of lipoids, lipid phosphoric acid (P_2O_5), and fat.

The tentative method for fat in baked cereal products³ is similar to the method for lipoids in flour and alimentary pastes, except that an

¹ *This Journal*, 1926, 9: 42.

² For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 86.

³ *Methods of Analysis*, A. O. A. C., 1925, 231.

ammoniacal extraction is made. The method for lipoids, which involves a neutral extraction, is supposed to give a more desirable extract, in that it is less likely to cause decomposition of the fat-like substances by the solvents. For the sake of uniformity, it was thought advisable to substitute the acid hydrolysis method for fat for the present one, and to insert the method for lipoids, provided these methods proved satisfactory for bread. No attempt was made to study the direct ether extraction of fat, since it has been shown conclusively that such an extraction is unsuitable for baked cereal products.

The methods used for lipoids and lipid phosphoric acid have been published¹. The details of the acid hydrolysis method are those given for this determination in alimentary pastes.

The results are given in the table.

TABLE 1.
*Collaborative results on lipoids, lipid phosphoric acid (P_2O_5),
and fat by acid hydrolysis.*

COLLABORATOR	LIPOIDS <i>per cent</i>	LIPID P_2O_5 <i>per cent</i>	FAT <i>per cent</i>
Bailey	4.73	0.050	4.40
	4.51	0.043	4.38
Bornmann	3.62	0.047	4.52
	3.62	0.035	4.52
Feldstein	4.00	4.40
Horst	4.645	4.32
	4.646	4.52
McRoberts	5.39	0.058	4.44
	5.29	0.054	4.29
Salinger	4.29	0.04	4.16
	4.17	0.04	4.21

COMMENTS OF COLLABORATORS.

J. H. Bornmann (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.).—I think the lipid and lipid P_2O_5 method is O. K., though I did not get close checks on the P_2O_5 . The fat by acid hydrolysis method appears to be satisfactory. Hydrolyzing in the tube is a good modification of the original.

R. L. Horst (U. S. Food, Drug and Insecticide Administration, New Orleans, La.).—All my blank determinations were negligible, so no corrections were necessary. No difficulties were experienced.

DISCUSSION.

The results for fat by acid hydrolysis are satisfactory. The method is simpler than the tentative method for baked cereal products and is considered to be in shape for adoption by the association.

The results for lipoids and lipid phosphoric acid (P_2O_5) are irregular, as they have been, to some extent, for all the cereal products tested in the

¹ *This Journal*, 1926, 9: 40.

past three years. The collaborators appear to have no difficulty in obtaining duplicate results which check well, but the agreement among some of the collaborators is poor. There is reason to believe that changes occur in the lipid content of the product, and this may account for some of the discrepancies encountered. The relatively coarse condition of the sample may be a source of error, though the associate referee has obtained good check results on samples of 20-, 40-, and 60-mesh fineness. The collaborators apparently experienced no difficulties in the manipulation. It is believed advisable to subject the method to a more thorough study.

RECOMMENDATIONS¹.

It is recommended—

(1) That the method for fat by acid hydrolysis in bread be adopted as tentative, and a comparative study of this method and the present tentative method for fat in baked cereal products be made.

(2) That study of the determination of lipoids and lipid phosphoric acid in bread be continued.

The report on milk solids on milk bread is given in the report on methods for bread analysis by L. H. Bailey, p. 392.

No report on rye flour in rye bread was given, as no associate referee was appointed.

REPORT ON EXPERIMENTAL BAKING TEST.

By M. J. BLISH (Agricultural Experimental Station, Lincoln, Nebr.),
Associate Referee.

Efforts to establish a standard laboratory baking test were actively indulged in during the past year, during which time the writer continued to serve two organizations in the same capacity, that is to say, as Chairman of the Committee on Standardization of the Experimental Baking Test for the American Association of Cereal Chemists, and as associate referee on the same project for the Association of Official Agricultural Chemists. Under these circumstances, the logical procedure is to present upon this occasion the report that was submitted at the recent annual meeting of the American Association of Cereal Chemists. That report has been published in full². The present report, therefore, may appro-

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 86.

² *Cereal Chem.*, 1928, 5: 158, 277.

priately be confined to the mere mentioning of certain items which are discussed in more detail in the published report.

A definite, fixed baking procedure has been formulated, presented in detail, and proposed as a tentative standard method. It is based upon principles and technic that were used and described by Werner¹. It was subjected to thorough discussion, study and verification by a qualified committee, and approved as a method that exceeds all other known or conceivable methods in the extent to which it meets the three following major requirements:

1. Strict adherence to the principle of fixed specifications, whereby the material to be tested is the only variable.
2. Usefulness in permitting the ascertainment of differences among flour characteristics, and the translation of these differences into terms of commercial utility.
3. Maximum simplicity and conservation of time, material, labor, and equipment.

As stated in the more detailed report, this method is one in which the foregoing requirements are fulfilled to a degree which can hardly be improved upon, except by the probable future development of better mechanical facilities, such as standard laboratory mixers and molders, respectively. The proposed method deals primarily with a "basic procedure", which is intended as the standard point of reference. Allowance is made for additional or supplementary procedures that are optional with the operator, and that, respectively, permit the varying of but one factor at a time.

The proposed method, which has also been published by this association², was adopted as a tentative standard procedure by the American Association of Cereal Chemists at its recent annual meeting.

In addition to the proposal of a definite and specific method of procedure, the published report discusses certain important matters bearing upon the ultimate *establishment* of the method as a standard procedure. Attention is especially directed to the fact that up to the present stage of the project it has not been possible to deal with the situation in accordance with the procedure which is generally prescribed and followed by the A. O. A. C. in the establishment of official methods. Thus, for instance, collaborative tests conducted in the customary and conventional manner have not given satisfactory results unless conducted in the same laboratory, although by different workers. Some reasons for this are as follows:

1. Lack of uniformity in laboratory equipment.
2. General lack of means for securing the necessary degree of precision in the control of environmental conditions, especially in regard to baking temperatures.

¹ *Cereal Chem.*, 1925, 2: 310.

² *This Journal*, 1920, 12: 41.

3. Multiplicity of factors involved, especially variations in yeast, and varying degrees of skill in molding and otherwise handling the dough.

4. Lack of an appropriate system for reporting results in terms of common understanding and interpretation.

It has been demonstrated, however, that different operators can secure acceptably concordant results when using the proposed method with the same laboratory equipment and materials.

The published report undertakes to answer some objections to the proposed test that have arisen in certain quarters, and it calls attention to numerous advantages that would ultimately accrue to all branches of the wheat industry through the establishment of a standard "quality" test.

While many admit the soundness of the principle of the fixed and definitely specified procedure as a necessary basis for any scientifically conducted baking test and grant that these principles are and have been habitually violated in the majority of cereal testing laboratories, they object strenuously to the proposed substitution of the commercial sized loaf by the small loaf made from 100 grams of flour. However, one of the items of the published report, entitled "A comparison of the proposed A. A. C. C. baking test and the commercial loaf (350 grams flour) test", by Bailey, Fifield, and Sherwood¹, conclusively indicates definite advantages in favor of the smaller loaf. Their conclusions, based upon a statistical analysis of more than 1000 individual test bakes, are that there is no indication that the small dough method is in any sense less satisfactory than the large dough method. In fact, such advantages as are evident are attached to the small dough method, particularly in the matter of variability. It is, of course, obvious that the small dough method permits of greater economy of material and of equipment necessary for the precise control of environmental conditions.

One of the most important phases of the entire project is the establishment of an appropriate system for reporting the results of baking tests. Since the test loaf constitutes the basis for judging the characteristics of the flour, it follows that the report must present a clear and adequate picture of loaf characteristics in terms of common understanding. The method of reporting must be strictly impartial, and as simple as possible.

The detailed report includes the proposal of a system involving the use of models or photographs representing various standard types of external and internal loaf characteristics, respectively. A standard report will then consist merely of specifying (by letter or number) the type to which the experimental loaf bears greatest resemblance, and indicating the *degree* of resemblance. The assumption is, of course, that each technologist will have in his possession a complete set of the standard models or photographs for reference.

¹ *Cereal Chem.*, 1928, 5: 287.

Other items included in the published report are "Possible causes for variations in collaborative reports" by Harrel¹, and some observations as to the application of the proposed test to soft wheat flours, by Whiting².

The situation may be briefly summarized by the statement that the project is being rapidly brought to a status in which collaborative tests can be conducted in accordance with the procedure which is conventionally employed in the promotion of standard or official tests. However, there are still some factors causing variation among individual laboratories that must be dealt with before this state of affairs is finally reached. One of the most serious of these factors, as has been especially noted and emphasized by Harrel, is the matter of variation in yeast quality. The work planned for the coming year contemplates, among other things, a study designed to ascertain the nature of variation among yeasts, both as to the different commercial brands and as to different lots of the same brand. More work is also needed in the matter of the adoption of a definite and uniform system for the reporting of results of laboratory baking tests.

The proposed test has been agreed upon by a Committee of the American Association of Cereal Chemists as the most suitable and appropriate available basis for the ultimate establishment of a standard laboratory baking test. The committee, which is composed of presumably well qualified representatives of the various important branches of the industry, has considered the problem from all obtainable viewpoints. It reached its definite conclusion only after more than two years of study, discussion, and critical verification. In addition to being the type of procedure best adapted to standardization, it has most successfully undergone the test of commercial and industrial application.

As previously stated, the proposed method has recently been adopted and approved as a tentative standard method by the American Association of Cereal Chemists. The associate referee recommends that it also be accepted as a tentative method by the Association of Official Agricultural Chemists³.

REPORT ON UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL IN ALIMENTARY PASTES.

By SAMUEL ALFEND (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.), *Associate Referee*.

Samples of patent flour (A) and egg noodles (B) were sent out for collaborative study. The noodles were ground to pass through a 20-mesh

¹ *Cereal Chem.*, 1928, 5: 296.

² *Ibid.*, 299.

³ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 87.

screen. The directions for analysis are given below. The results are given in Tables 1 and 2.

DIRECTIONS FOR ANALYSIS.

UNSAAPONIFIABLE MATTER.

Extract the lipoids from Samples A and B according to the official method¹. Determine the unsaponifiable matter in the extracted lipoids by the modified F. A. C. method².

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

Place 20 grams of Sample B in an 8-ounce nursing bottle, add 100 cc. of water from a pipet, shake the bottle vigorously to prevent lumping of the sample, and add exactly 100 cc. more of water. Shake the stoppered bottle mechanically or by hand for 1 hour. (The temperature of the water should not exceed 30°C.) Proceed according to Method 1 or 2 below.

Method 1.—Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. (The filtrate should be practically clear.) Pipet 50 cc. of the filtrate into a 200 cc. nursing bottle. Add 0.6 gram of sodium chloride and dissolve. Add 0.2 gram of ignited asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Let stand overnight, then centrifugalize to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off and wash with two 20 cc. portions of 40 per cent alcohol, in each case shaking, centrifugalizing, and decanting. If the liquid is not free of suspended matter, filter through a thin asbestos pad (0.1–0.15 gram) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos in the nursing bottle to a Kjeldahl flask with the aid of a stream of water, add to it the mat in the Gooch crucible, and determine the nitrogen by the Kjeldahl-Gunning-Arnold method³, using about 20 cc. of 0.1 *N* acid to receive the distillate. Make a blank determination on the reagents and on the asbestos.

Method 2.—Centrifugalize and filter as in Method 1. Determine nitrogen in 50 cc. of the filtrate as directed in the official method³, distilling the ammonia into 30 cc. of 0.1 *N* acid. Run a blank on the reagents. Pipet off 100 cc. of the above filtrate into a 200 cc. volumetric flask, add 1.2 grams of sodium chloride, dissolve, add 70 cc. of 95 per cent alcohol, mix carefully to avoid foaming, cool to room temperature, and make up to volume with 40 per cent alcohol. Shake well and allow to stand overnight to permit complete precipitation of the albumin. Pipet off the supernatant solution and filter through an asbestos pad in a Hirsch funnel or Gooch crucible, using light suction. Determine nitrogen in 75 cc. of the filtrate as above. Distil the ammonia into 20 cc. of 0.1 *N* acid. Run blank determinations on the reagents. Multiply the results for nitrogen in 75 cc. of the 40 per cent alcohol filtrate by 4/3, and subtract the product from the nitrogen contained in 50 cc. of the water extract, to determine the amount of the water-soluble protein-nitrogen precipitable by 40 per cent alcohol in 50 cc. of the aqueous extract.

COMMENTS OF COLLABORATORS.

J. H. Bornmann (U. S. Food, Drug and Insecticide Administration, Chicago, Ill.).—I prefer Method 1 for nitrogen precipitable by alcohol. I think it is less work.

Leonard Feldstein (U. S. Food, Drug and Insecticide Administration, Denver, Colo.).—Neither of the two methods described for water-soluble nitrogen showed any superiority

¹ *This Journal*, 1926, 9: 40.

² *Ibid.*, 45.

³ *Methods of Analysis*, A. O. A. C., 1925, 8.

over the official method for flour. In Method 1 difficulty was experienced in getting all the precipitate out of the bottle into the Kjeldahl flask, a fairly large amount of water being required; in the subsequent digestion there was a decided tendency to bump and foam and several determinations were lost. It was necessary to watch each digestion very carefully, and this seems a waste of time. If all the precipitate is collected on a Hirsch funnel and the precipitate is digested as in the method for flour, no foaming is noticeable, and there is very little bumping. The same difficulty of bumping and foaming was experienced in Method 2 in digesting the alcoholic solution. Method 2 has nothing to recommend it. Why make two nitrogen determinations when one will suffice?

*L. H. McRoberts (U. S. Food, Drug and Insecticide Administration, St. Louis, Mo.).—*In extracting the lipoids from Samples A and B for unsaponifiable matter the ether solutions were decanted through a filter containing a pledget of cotton into a 250 cc. Erlenmeyer flask containing some bits of broken glass. The material was then contained in the proper vessels for the subsequent saponification. The petroleum ether available for this determination contained considerable residue (350 cc. contained 0.0183 gram). The final residue gained weight when the drying was continued for more than 1 hour.

In the determination of water-soluble protein in egg noodles it was difficult to obtain a clear filtrate following the water extraction to obtain total water-soluble nitrogen. The combination of centrifugalization followed by filtration through a thin asbestos pad did not remove a very fine colloidal suspension. In the determination according to Method 2 a clean separation was not possible following the precipitation with 40 per cent alcohol.

DISCUSSION.

The results on unsaponifiable matter are unsatisfactory, particularly for flour, as they have been in the collaborative work of the last two years. Since the method is considered the best available, for reasons discussed in previous reports, it is recommended that no further work be done upon it.

The official method for water-soluble nitrogen in flour¹ is satisfactory for flour and for water noodles, but it is unsatisfactory for substances which have a larger proportion of albumin, such as egg noodles. The methods for water-soluble nitrogen sent to the collaborators were based upon previous collaborative experience.

Method 1 gives slightly more uniform results than Method 2 and is preferred by most of the collaborators, although the associate referee has found the latter method more convenient. The average results by the two methods are practically identical.

RECOMMENDATIONS².

It is recommended—

(1) That the tentative (F. A. C.) method for unsaponifiable matter in flour and alimentary pastes³ be made official.

¹ *This Journal*, 1926, 9: 40.

² For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 87.

³ *Ibid.*, 1928, 11: 37, 39.

(2) That the method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes, described in this report as Method 1, be adopted as tentative.

(3) That the official method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes¹ be dropped.

TABLE 1.
Collaborative results.

COLLABORATOR	WATER-SOLUBLE PROTEIN-NITROGEN		UNSATURIFIABLE MATTER	
	Method 1 <i>per cent</i>	Method 2 <i>per cent</i>	Flour (A) <i>per cent</i>	Egg Noodles (B) <i>per cent</i>
Bailey	0.14	0.16	0.39	0.38
	0.15	0.12	0.40	0.29
Bornmann	0.20	0.18		
	0.19	0.19		
Feldstein	0.191	0.185		
Horst	0.163	0.161	0.31	0.67
	0.165	0.163	0.33	0.71
McRoberts	0.19	0.29	0.39
	0.24		0.25	0.39
Salinger	0.21	0.24	0.12	0.32
	0.21	0.24	0.14	0.36

REPORT ON MOISTURE IN ALIMENTARY PASTES.

By S. C. ROWE (Food, Drug and Insecticide Administration, Washington, D. C.), *Associate Referee*.

The vacuum method² and air oven method³ having been adopted as official for moisture in flour, it was deemed desirable this year to study the methods collaboratively for the determination of moisture in alimentary pastes.

A 4 ounce bottle of ground egg noodles sealed with paraffin was submitted to collaborators with the request that moisture be determined by each method. The results obtained are given in the table. The difference in the average moisture content determined by the two methods, 0.01 per cent, is very gratifying since the collaborators were located in widely separated sections of the country.

Although this is the first year that collaborative work has been undertaken on the determination of moisture in alimentary pastes by these methods, it is felt that they should be adopted as official. The results obtained this year, the critical study previously made on the methods in connection with flour, and a consideration of the composition of alimentary pastes would appear to be sufficient reasons to justify this action.

¹ *This Journal*, 1926, 9: 431.

² *Ibid.*, 39.

³ *Ibid.*, 40.

Results obtained by collaborators on samples of A. O. A. C. Noodles (1928).

(Expressed in percentage.)

COLLABORATORS	VACUUM METHOD						ONE HOUR AT 130°C.					
	1	2	3	4	5	Average	1	2	3	4	5	Average
George Daughters F. D. & I. Adm'n San Francisco, Calif.	7.52	7.57	7.50			7.53	7.47	7.37	7.37			7.40
Leonard Feldstein F. D. & I. Adm'n Denver, Colo.	7.40	7.41 (Temp. boiling H ₂ O 94.5°C.)	7.45			7.42	Not reported. Oven not heated uniformly with Bunsen burners					
Leslie Hart F. D. & I. Adm'n Chicago, Ill.	7.59	7.52	7.55	7.58		7.56		7.55	7.60	7.59		7.58
R. L. Horst F. D. & I. Adm'n New Orleans, La.	7.52	7.54	7.52			7.53	7.45	7.49	7.46	7.47	7.45	7.46†
H. I. Macomber F. D. & I. Adm'n New York, N. Y.	7.20	7.20	7.18			7.19*	7.51	7.53	7.48			7.51
H. R. Smith F. D. & I. Adm'n Baltimore, Md.	7.46	7.46	7.47			7.46	7.48	7.54	7.60			7.54
S. C. Rowe	7.59	7.57	7.59	7.58		7.58	7.59	7.60	7.59	7.57		7.59
Maximum Minimum Average						7.58 7.42 7.51						7.59 7.40 7.52

* Not included in average. Pressure 2-4" too high.
† Not included in average. Heated 2 hours at 130°C.

RECOMMENDATIONS¹.

It is recommended—

- (1) That the vacuum method for the determination of moisture in flour be adopted as official for this determination in alimentary pastes.
- (2) That the air oven method for the determination of moisture in flour be adopted as official for this determination in alimentary pastes.

GREETINGS FROM THE DEPARTMENT OF AGRICULTURE.

By A. F. WOODS, Director of Scientific Work.

I am sorry that Secretary Jardine could not be with you in person this afternoon to extend the official greeting of the Department of Agriculture, but he is hard at work helping to select a pilot for our great Ship of State for the next four years. I have the honor to present his greetings to you and his best wishes for your continued success in the great field of service to humanity in which you are engaged.

Your work stands at the foundation of our program for purity and honesty in foods, drugs, animal feeds, fertilizers, insecticides and fungicides, and a host of other things that have important relation to our general welfare. Not many outside of the official family know how extensive are your researches and what an important relation they have to our general well-being.

We are now well started in a new era of more exact agricultural science, especially from the chemical standpoint. The new developments in chemistry have opened up new fields and required the reexamination of old fields from the standpoint of the newer and more exact methods today available.

The history of your association is the history of advance and improvement in chemistry applied to agriculture. While you must necessarily be cautious and careful, you have always led in such forward movements. Today on the eve of the chemical revolution you stand on the advanced firing line in making chemistry more than ever the benefactor of man. We are looking to you for guidance in this field.

Recently, as you know, in the Department of Agriculture we have regrouped some of our chemical work to secure, we hope, greater efficiency in our regulatory and research groups. The regulatory functions of the old Bureau of Chemistry now constitute a separate group—the Food, Drug and Insecticide Administration. The chemical determination and research necessary to enforce the regulatory laws are retained in that group, and all the thought and energy of the regulatory chemists are devoted to that end.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 87.

Under the designation of the Bureau of Chemistry and Soils the agricultural research units are grouped into a great chemical research organization, including chemical technology investigations and chemical engineering, fertilizers and nitrogen fixation, and soils. Very close cooperative relations are maintained between the units of this research group and the regulatory group. The increased efficiency and interest in both groups is evidence of the success of the move.

The public is more and more appreciating the valuable service rendered by both these groups and is demanding their better financial support. The industries affected are also strongly back of the work because they know that their success depends on accurate scientific information and protection from adulterators and those who would misrepresent their products.

To you we look for methods of improving these services. I can assure you that the Department of Agriculture desires to cooperate with you fully and cordially. We are always glad to get your suggestions and constructive criticism, and we wish you every success in your great work.

No report on beers, wines and distilled liquors was given by the referee.

No report on specific gravity and alcohol was given by the referee.

No report on vinegars was given by the referee owing to his late appointment.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE (U. S. Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

Complaints were received that the official colorimetric method¹ for citral in oils and extracts of lemon and orange is unworkable in many instances due to the development of an interfering blue or green color. The referee decided, therefore, in view of the importance of the subject, to investigate the method of Parker and Hiltner², a modification of the official method designed to prevent the formation of interfering colors, and for the present to defer work on the steam distillation method for determination of oils in non-alcoholic flavors.

In the proposed modification, a metaphenylenediamine hydrochloride-oxalic acid solution is substituted for the metaphenylenediamine hydro-

¹ *Methods of Analysis*, A. O. A. C., 1925, 354-5.

² *J. Ind. Eng. Chem.*, 1918, 10: 608.

chloride solution used in the official method. A detailed description of the modified procedure has been published¹.

J. B. Wilson prepared five lemon extracts containing 5-6 per cent by volume of oil dissolved in alcohol, 85 per cent by volume. Three of the lemon oils were old (Nos. 1, 2, and 3) and two (Nos. 4 and 5) were alleged to be new oils. The extracts were analyzed for content of citral by four collaborating chemists, both the official and the modified official methods being used. The results obtained are given in Table 1.

TABLE 1.

Colorimetric determination of citral in lemon extracts.*

SAMPLE	C. H. BADGER		W. F. CLARKE		A. E. MIX		J. B. WILSON	
	Official Method	Modified Official Method	Official Method	Modified Official Method	Official Method	Modified Official Method	Official Method	Modified Official Method
	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Off color†	0.13		0.15	Off color†	0.15	Off color†	0.16
2	Off color†	0.23		0.23	Off color†	0.23	Off color†	0.23
3	0.16	0.17		0.18	0.17	0.17	0.14	0.16
4	0.23	0.22		0.23	0.25	0.23	0.20	0.21
5	0.17	0.23		0.21	0.18	0.23	0.18	0.22

* Made by dissolving about 5 cc. of lemon oil in about 95 cc. of alcohol, 85 per cent by volume. Samples 1, 2, and 3 were made up from old oils, while Samples 4 and 5 were made from oils which were alleged to be freshly prepared.

† The interfering color was green. When some very old metaphenylenediamine hydrochloride was used, none of the collaborators was able to report results by the official method because of interfering color reactions. All the results reported on the modified official method were obtained by the use of the old reagent.

DISCUSSION OF RESULTS.

The data in Table 1 show (1) that the official method gave unsatisfactory results on several of the samples because of formation of interfering colors, and (2) that the modified method gave results that checked very closely. There is some evidence that when the oils and reagent are fresh, there is little or no interference from off colors, but that when the reagent or oils are old, the colors obtained cannot be compared. When the official method was tested by the referee in 1910², a number of the collaborators complained of the formation of interfering colors. Of course, the modified method was not available at that time. The question may be asked as to how closely the results obtained by the modified method represent the actual content of citral in the samples, and it can only be stated that it has not been shown that the actual content of citral can be determined by either the official or the modified official

¹ *This Journal*, 1929, 12: 48.

² U. S. Dept. Agr. Bur. Chem. Bull. 187, p. 64.

method. From theoretical considerations, it appears more probable that these methods merely give a somewhat low figure for total aldehydes, probably because the reaction does not go to completion, rather than a true figure for citral content. The aldehyde radical, which reacts with the reagent, is common to both citral and the other aldehydes that are present in oils of lemon and orange.

However this may be, the referee believes that these methods should not be abandoned until such time as the total aldehyde content calculated as citral, instead of the citral content, is made the basis of judging oil and extract of lemon used for food purposes, as is now being done in the case of oil of lemon for drug purposes. In the meantime, it would be desirable to obtain an alternative method for the determination of total aldehydes in lemon and orange extracts, since the fuchsin-sulfite method, while accurate, is long and cumbersome. A gravimetric method has been proposed by Radcliffe and Swann¹. Some preliminary experiments made in the Water and Beverage Unit indicate that this method is worthy of further investigation.

Under the circumstances, it is recommended that the official method for citral in lemon and orange extracts be withdrawn and that the modified method described above be made official.

Both the official Kleber method and the official Hiltner method appear under the heading "Lemon and Orange Oils—Citral", in *Methods of Analysis*², although it is recognized that the Kleber method gives total aldehydes and that the Hiltner method gives much lower results than the Kleber method. The following statement from one of the field stations of the Food, Drug and Insecticide Administration is of interest in this connection:

* * * The work has shown that if the citral in lemon oil is determined by the Hiltner method, the pure lemon oil will show a minimum of 3.60 per cent to a maximum of 4.15 per cent, whereas, by the Kleber method, you have a minimum of 4.09 per cent to a maximum of 5.30 per cent. It is obvious that the manufacturer who uses the Kleber method for determining his citral and who makes up his terpeneless extract of lemon as defined under definition No. 10 by the results obtained by that method will have an advantage over the manufacturer who uses the Hiltner method and uses the figures obtained in that way for manufacturing his extract * * *.

* * * The records show that citral in orange oil determined by the Hiltner method had a minimum of 0.29 per cent to a maximum of 0.72 per cent. The Kleber method gave a minimum of 1.80 per cent to a maximum of 2.70 per cent * * *.

The above data, taken in connection with the fact that the Kleber method depends upon a reaction which is general for aldehydes, would seem to make it desirable to put the Kleber method under the heading, "Lemon and Orange Oils—Total Aldehydes". Two alternative official methods would then be available for total aldehydes in the oils, one of

¹ The Perfumery and Essential Oil Record, 19: 2, 47-51.

² *Methods of Analysis*, A. O. A. C., 1925, 355.

which, the fuchsin-sulfite method, would probably give results which would be but little higher than those obtained by the Kleber method. However, at this time the referee does not feel warranted in recommending this change, but would like to have the subject discussed with a view to making the change next year.

RECOMMENDATIONS¹.

It is recommended—

(1) That the steam distillation method for the determination of oils of lemon, orange, and limes in corn and cottonseed oils and in mineral oil, described in the report of the referee last year², be adopted as official (final action).

(2) That the colorimetric method for the determination of small quantities of anthranilic acid ester, described in the report of the referee last year², be adopted as official (final action).

(3) That the gravimetric method for the determination of large quantities of anthranilic acid ester, described in the report of the referee last year², be adopted as official (final action).

(4) That the official method for citral in lemon and orange extracts be withdrawn and that the method described in this report, which is a modification of the official method, be adopted as official (first action).

(5) That the study of the method referred to in Recommendation 1 be continued with a view to extending its use to other non-alcoholic flavors.

(6) That an effort be made to obtain a method for total aldehydes in lemon and orange extracts which the association will be warranted in adopting as an alternative official method.

REPORT ON MEATS AND MEAT PRODUCTS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.),
Referee.

A cooperative study of the method for the detection and determination of added water in sausage and similar meat food products was made this year. Test samples were prepared by grinding and thoroughly mixing a quantity of commercially prepared meat food product and dividing it into three portions. One portion designated as Sample A was used without further treatment; two portions of 720 and 640 grams, respectively, were mixed with 80 and 160 cc. of water and designated as Samples B and C. The water was incorporated by thorough mixing. The respective composition of the three samples sent to each collaborator, therefore, was as follows:

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 83.

² *This Journal*, 1928, 11: 45-48.

TABLE 1.
Results reported by collaborators.

COLLABORATOR	SAMPLE	TOTAL WATER	PROTEIN (N \times 6.25)		ADDED WATER
			On moist sample	On dry basis	
Frederic Fenger Armour & Co. Chicago, Ill.	A	<i>per cent</i> (1) 63.19 63.59 63.54	<i>per cent</i> 14.68 14.57 14.92	<i>per cent</i>	<i>per cent</i>
		Av. 63.44	Av. 14.72	40.26	4.56
		(2) 63.09 62.99 63.15			
	B	Av. 63.08		39.87	4.20
		(1) 66.49 67.12 66.69	13.59 13.39 13.79		
		Av. 66.77	Av. 13.59	40.89	12.41
	C	(2) 66.56 66.50 66.80			
		Av. 66.61		40.40	12.25
		(1) 70.70 70.72 70.65	12.00 11.79 11.89		
		Av. 70.69	Av. 11.89	40.56	23.13
		(2) 70.41 70.62 70.41			
		Av. 70.48		40.27	22.92
S. W. Wiley Wiley & Co. Baltimore, Md.	A	63.21	14.67	39.87	4.53
	B	66.55	(3) 12.86	(3) 38.44	(3) 15.11
	C	70.02	12.07	40.26	21.74
David Edelman Bur. Animal Industry New York, N. Y.	A	63.46 63.44 63.45	15.05 15.08 14.91	41.18 41.18 40.79	3.26 3.12 3.81
		66.29 66.40 66.29	13.78 13.74 13.65	40.88 40.89 40.49	11.17 11.44 11.69
		69.91 70.14 70.31	12.20 12.32 12.32	40.51 41.22 41.49	21.11 20.86 21.03
	B				
	C				

TABLE 1—Continued.
Results reported by collaborators.

COLLABORATOR	SAMPLE	TOTAL WATER	PROTEIN (N \times 6.25)		ADDED WATER
			On moist sample	On dry basis	
H. R. McMillin Bur. Animal Industry Washington, D. C.	A	<i>per cent</i> 63.39 63.34	<i>per cent</i> 15.05 14.91	<i>per cent</i> 41.11 40.68	<i>per cent</i> 3.19 3.70
		Av. 63.36	Av. 14.98		Av. 3.44
	B	66.43 66.47	13.67 13.69	40.72 40.83	11.75 11.71
		Av. 66.45	Av. 13.68		Av. 11.73
	C	70.28 70.65	12.19 12.20	41.01 41.56	21.52 21.85
		Av. 70.47	Av. 12.20		Av. 21.67
P. A. Sigler Bur. Animal Industry Washington, D. C.	A	63.38 63.32 63.45	14.82 14.98 14.80	40.46 40.57 40.49	4.10 3.40 4.25
	B	66.77 66.94	13.55 13.53	40.78 40.92	12.57 12.82
	C	70.97 70.74	11.92 11.93	40.37 40.77	23.29 23.02
Wm. C. Owens Bur. Animal Industry San Francisco, Calif.	A	63.38 63.36	15.07 15.22	41.15 41.48	3.08 2.46
	B	66.28 66.46	(3) 14.15 (3) 14.12	(3) 41.96 (3) 42.10	(3) 9.68 (3) 9.96
	C	69.61 69.63	(3) 13.25 (3) 13.20	(3) 43.60 (3) 43.46	(3) 16.61 (3) 16.83

(1) Determined by proposed method.

(2) Determined by official method, Chapter IX, 2.

(3) Rejected.

Sample A.—Prepared meat food product containing an unknown proportion of added water.

Sample B.—Prepared meat food product containing an unknown proportion of previously added water and 10 per cent water added in preparation.

Sample C.—Prepared meat food product containing an unknown proportion of previously added water and 20 per cent water added in preparation.

Collaborators were requested to test each sample for moisture, protein, and added water in accordance with the methods submitted. These methods have been published¹.

The results are given in Table 1.

¹ *This Journal*, 1929, 12: 43.

The results reported by the six collaborators are in excellent agreement, as shown in Table 2, which gives the maximum, minimum, and average of the accepted results.

TABLE 2.
Maximum, minimum, and average of accepted results.

	SAMPLE	TOTAL WATER	PROTEIN (N \times 6.25)		ADDED WATER
			On moist sample	On dry basis	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Maximum	A	63.59	15.22	41.48	4.56
Minimum		63.19	14.57	39.87	2.46
Average		63.37	14.90	40.65	3.77
Maximum	B	66.94	13.79	40.89	12.82
Minimum		66.28	13.39	40.78	11.17
Average		66.56	13.63	40.82	12.07
Maximum	C	70.97	12.32	41.56	23.29
Minimum		69.61	11.79	40.26	20.86
Average		70.29	12.07	40.75	22.14

The results marked as rejected in Table 1 were excluded because calculation of the protein to the dry basis indicated error. Since the protein constituent of all three samples was the same, the results of the protein determination, when calculated to the dry basis, should have been concordant. By computation from the results reported, 40.75 per cent was assumed as the correct value, and all results that varied from that value by more than 1 per cent were rejected.

Although 10 per cent of water was added to Sample A to make Sample B and 20 per cent to make Sample C, the percentages of added water found in these samples are less than 10 and 20, respectively. The average recovery in Sample A was approximately 87 per cent and in Sample C it was approximately 93 per cent. Loss of moisture in the process of mixing appears to be the most probable explanation of the discrepancy. Particular care was taken to secure thorough mixing, and neither sample was weighed after mixing to determine the loss involved in the process. The consistency of the analytical results, particularly the protein calculated to the dry basis, appears to exclude other possible causes of discrepancy.

Determinations of protein were also made by H. R. McMillin, who used a rapid-boiling, short-digestion method¹. The results, together with the corresponding determinations of total water and added water, are shown in Table 3.

¹ *This Journal*, 1928, 11: 408.

TABLE 3.

Water, protein, and added water determined by rapid-boiling, short-digestion method.

(Analyst—H. R. McMillin.)

SAMPLE	TOTAL WATER	PROTEIN ($N \times 6.25$)		ADDED WATER
		On moist sample	On dry basis	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A	63.49	14.84 14.81	40.59	4.21
B	66.82	13.58 13.56	40.89	12.54
C	70.66	12.11 12.12	41.01	22.18

RECOMMENDATIONS¹.

It is recommended—

(1) That the method studied be adopted as a tentative method for the determination of added water in sausage and similar meat food products.

(2) That the words "In the Kjeldahl-Gunning methods digest with sulfuric acid for at least 4 hours; in the Kjeldahl-Gunning-Arnold method, for 2 hours after the mixture has become clear", be deleted from the official method for total nitrogen in meats². This is a recommendation for final action on a change in an official method. The method as changed will read as follows: "Proceed as directed on p. 7, 19, or p. 8, 22 or 24, using about 2 grams of the fresh sample". The effect of this change will be to leave the directions for digestion the same as in Chapter I, 19, 22, or 24, p. 6.

(3) That consideration be given by the Referee on Nitrogen to the rapid-boiling, short-digestion method.

REPORT ON SEPARATION OF MEAT PROTEINS.

By W. S. RITCHIE (University of Missouri, Columbia, Mo.), *Associate Referee*.

The methods to be discussed are a somewhat radical departure from the methods now appearing in *Official and Tentative Methods of Analysis*, A. O. A. C. The data upon which they are based will be published elsewhere³.

The soluble proteins, according to the present methods, are to be extracted with cold water. As indicated by Moulton⁴, not only is albumin

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 83.

² *Methods of Analysis*, A. O. A. C., 1925, 237.

³ *J. Am. Chem. Soc.*, 1929, 51: 880.

⁴ *This Journal*, 1922, 6: 76.

extracted by water, but some globulin is also removed by the dilute salt solution formed by the addition of water to the meat. It seemed advisable, therefore, to choose an extracting solution that would extract the globulin as well as the albumin.

The following solutions were investigated to this end: 10 per cent sodium chloride, 0.9 per cent sodium chloride, 10 per cent ammonium chloride, 10 per cent ammonium sulfate, 5 and 10 per cent magnesium sulfate, 10 per cent lithium sulfate, and a mixture of potassium dihydrogen phosphate and disodium hydrogen phosphate having a pH of 7.0.

The ammonium solutions were rejected because of the difficulty in determining nitrogen in the presence of so much ammonia. It may be said that 10 per cent ammonium chloride gave the highest extraction obtained, 82 per cent.

The phosphate mixture gave an extraction of about 58 per cent of the total nitrogen, but was discarded for the present at least.

The lithium sulfate available gave an acid reaction in solution, and it was not deemed advisable to use it.

The magnesium sulfate did not give a high percentage of extraction and was thought to present difficulties in later shifting of the pH of the solution. It was discarded.

The 0.9 sodium chloride was not considered, as it gave a very low percentage of extraction.

The 10 per cent solution of sodium chloride gave a higher percentage of extraction than any solution, excepting the 10 per cent ammonium chloride, and it was possible to titrate this solution without difficulty. For these reasons, it seemed that 10 per cent sodium chloride was best suited for the purpose. The pH of this solution was always brought to 6.00 ± 0.1 .

Preparation of the extract.—One hundred grams of finely ground fresh meat was divided among four 200 cc. centrifuge bottles, and about 100 cc. of extracting solution was added to each bottle. The bottles were shaken, allowed to stand a short time in an ice and salt mixture, and centrifuged, and after the supernatant liquid had been poured into 2 liter flasks, they were packed in an ice and salt mixture. This procedure was repeated until extraction was complete, as evidenced by a lack of precipitate on heating a small portion and the addition of trichloroacetic acid to 2.5 per cent. The extract was filtered through cellucotton on a Büchner funnel. A solution of protein so prepared was free of any suspended material.

Separation of the proteins.—The present A. O. A. C. methods precipitate globulin by half saturation with zinc sulfate. It was the experience of the referee, however, that results obtained with this solution did not agree with those obtained by other methods. The following salts were

used in precipitating globulin: Magnesium sulfate, lithium sulfate, sodium chloride, ammonium sulfate, and zinc sulfate.

Saturation with sodium chloride and magnesium sulfate and half saturation with ammonium sulfate gave approximately the same result. Lithium sulfate could not be used, as the material at hand was too acid in solution. Ammonium sulfate was not used, because of the disturbing influence of so much ammonia. Saturation with sodium chloride was selected as a matter of convenience and because the extraction had been made with this solution.

It was soon very evident that the pH of the solution was an important factor in the separation of a protein by saturation with sodium chloride. Separations were made at a pH of 4, 5, 6, 7, and 8. Results obtained at pH 4 and 5 gave evidence that more than just globulin was being thrown down. Comparable results were obtained at pH 6, 7, and 8, although the condition of the precipitates at pH 7 and 8 made it necessary to eliminate them. Precipitates so obtained were gelatinous in character and hard to filter and wash.

Separation of globulin by saturation with sodium chloride.—Samples of 100 cc., in triplicate, were placed in 400 cc. beakers and brought to the pH desired by adding the quantity of 0.05 *N* hydrochloric acid or 0.05 *N* sodium hydroxide found necessary by previous titration. Sufficient sodium chloride was then added to saturate the solutions. (A large excess is to be avoided.) These were allowed to stand two or more hours with occasional stirring. It is necessary to stir during the addition of the sodium chloride. The precipitates were filtered and washed by decantation, 10 per cent sodium chloride of the proper pH being used. After five or more washings, the precipitates were transferred to the filter and allowed to drain. Nitrogen determined in the precipitate was taken as a measure of globulin.

Separation of albumin.—The filtrate from the globulin determination was heated to boiling, and trichloroacetic acid to 2.5 per cent was added. Nitrogen in the precipitate thus formed was indicative of the albumin present.

Non-protein-nitrogen.—Nitrogen in the filtrate from the albumin determination was taken as a measure of the non-protein-nitrogen present. It was obvious that a true value for albumin is gained only when proper separation of the globulin has been effected. Great care must be exercised in the determination of non-protein-nitrogen in the presence of so much sodium chloride. The sulfuric acid must be added slowly, and care must be taken that sufficient acid is added. Digestion must be carried on at a moderate pace, and it is necessary that there be enough acid to keep the contents of the flask liquid.

The referee also considered it wise to attempt to get similar results for globulin by dissimilar methods. For this purpose, dialysis and

exposure to ultra violet rays were used. Dialyzing cells were made of cork rings coated with paraffin. Parchment paper was clamped across the rings, any possible opening being closed with paraffin. The samples were introduced through holes in the ring, which later were stoppered. A glass tube in the cork ring took care of any pressure changes within. Toluene and thymol served as preservatives.

Separation of globulin by dialysis.—Samples of 100 cc. or 150 cc. were introduced into each of four cells, and the preservatives were added. The cells were sealed and dialyzed for 24 hours against cooled tap water, 24 hours against cooled distilled water, and 24 hours against water of pH 4, 5, 6, and 7. The precipitate formed was washed into 200 cc. centrifuge bottles, separated, washed by decantation, and finally transferred to the filter. Nitrogen was determined. The filtrates and washings were treated for albumin and non-protein-nitrogen as previously described. It is evident that the pH of the solution against which the cells were dialyzing during the last 24 hours had no apparent effect on the result. The non-protein-nitrogen fraction by this method obviously would be low.

Separation of globulin by ultra violet rays.—Aliquots of 50 cc. in petri dishes were exposed to the rays of a mercury-quartz arc at a distance of 12 inches from the arc. During the exposure the samples were stirred by a mechanism that moved the platform on which the dishes rested. The dishes and samples were weighed during the exposure, and the weight was kept constant by the addition of water. This process eliminated any effect of a higher concentration of sodium chloride. When precipitation was complete, the precipitate was washed into 100 cc. centrifuge tubes and separated. The supernatant liquid was poured through filters, and the precipitate was washed by decantation. After four washings the precipitate was transferred to the filter and allowed to drain. Nitrogen in the precipitate was taken as a measure of globulin separated by this method. Albumin and non-protein-nitrogen were determined in the filtrates as previously described.

It was evident that both the length of exposure and the pH of the solution were important factors. At pH units lower than 5.4, protein, other than globulin, was apparently precipitated. At pH units higher than 6.0 the globulin was incompletely thrown down. Likewise, too short an exposure gave incomplete precipitation. By experience it was learned that exposure for about 5 hours at pH units from 5.6 to 5.8 gave results agreeing among themselves and with results obtained by other methods.

Table 1 shows results that might be expected when the methods described are used.

The samples were obtained from the eyes of the 9th, 10th, and 11th ribs of steers killed at this Experiment Station in connection with a local project.

SUMMARY.

(1) Methods for the extraction and separation of globulin, albumin, and non-protein-nitrogen are described.

(2) Globulin is separated by saturation with sodium chloride, exposure to ultra violet rays, and dialysis to about the same extent.

(3) Albumin determined in the filtrates from the globulin separations in the same extract are of about the same order of magnitude. The same is true of non-protein-nitrogen.

RECOMMENDATIONS¹.

It is recommended—

(1) That further work be done concerning the extraction of these fractions and their separation by these methods.

(2) That the method of Howe² be compared with these methods.

(3) That collaborative work be done when possible in connection with Recommendations 1 and 2.

TABLE 1.
Results of analysis.

Steer No.....	136	19	503
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total nitrogen in meat.....	3.632	3.441	3.501
Total nitrogen in extract.....	2.021	2.025	2.227
Percentage of extraction.....	55.6	58.9	63.6
GLOBULIN*.			
Precipitated by NaCl.....	52.6	46.6	51.3
Precipitated by ultra violet rays.....	51.7	55.1	46.7
Precipitated by dialysis.....	50.4	52.4	47.7
ALBUMIN*.			
In NaCl filtrate.....	28.5	35.0	28.7
In ultra violet rays filtrate.....	21.5	25.2	30.0
In dialysis filtrate.....	25.1	27.7	26.3
NON-PROTEIN-NITROGEN*.			
In NaCl filtrate.....	26.1	22.4	15.5
In ultra violet rays filtrate.....	25.2	19.0	24.5
In dialysis filtrate.....	13.6	10.0	13.1

* Calculated in terms of extracted nitrogen.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 83.

² *J. Biol. Chem.*, 1924, 61: 493.

REPORT ON GELATIN.

By E. H. BERRY (Food, Drug and Insecticide Administration, Chicago, Ill.), *Referee*.

The last report on gelatin was made in 1926¹. At that time the present tentative method was studied in comparison with a so-called proposed method. The results obtained by the collaborators were far from satisfactory. Especially was this true regarding copper. Accordingly, the referee was instructed to make a further study:

(1)—of the method of preparation of the sample by ashing as compared with that by acid hydrolysis,

(2)—of the determination of copper for the purpose of developing a more satisfactory method than now available,

(3)—of the precipitation of zinc in formic acid solution.

The referee regrets that he was unable to make a report in 1927 and also that time has not permitted a more extensive study of, and search for, a more satisfactory method for the determination of copper in the small quantities usually found in gelatin and other food products.

Two collaborators were secured this year: R. M. Mehurin, Bureau of Animal Industry, Department of Agriculture, Washington, D. C., and Leslie Hart, Chicago Station, Food, Drug and Insecticide Administration. Two samples of gelatin were submitted to them with the request that copper and zinc be determined by the methods specified below. Sample A contained approximately 90 parts per million of copper and 300 parts per million of zinc; Sample B contained 30 parts of copper and 100 parts of zinc.

COPPER.

A. O. A. C. Tentative Method.

This method has been published².

ZINC.

A. O. A. C. Tentative Method.

This method has also been published².

COPPER.

Proposed Methods.

Ash 50 grams of gelatin in a platinum or porcelain dish of about 150 cc. capacity. (The temperature should be such that the muffle presents a barely visible red when the material is placed in it. This temperature should not be exceeded at any time.) As soon as volatile matter begins to come off, ignite the evolved gases, and keep them burning by holding the door of the furnace partly open. (The volatile matter will be off in about 10 minutes and if kept ignited but little odor will result. The ashing may

¹ *This Journal*, 1926, 9: 458.

² *Methods of Analysis*, A. O. A. C., 1925, 256.

be hastened by leaching the well-charred mass with water.) Moisten the ash with water, add approximately 5 cc. of concentrated hydrochloric acid, and evaporate to dryness. Dissolve in 10 cc. of hydrochloric acid (1 + 1) and 40 cc. of water and filter. Neutralize the filtrate with ammonium hydroxide, using methyl orange, and add sufficient excess to precipitate all iron and aluminum and assure solution of the copper and zinc. Heat the solution to about 80°C. Filter and wash the precipitate with a 3 per cent solution of ammonium chloride containing about the same quantity of free ammonium hydroxide as the original solution. Dissolve the precipitate with dilute hydrochloric acid and reprecipitate and filter as before. Neutralize the combined filtrates with hydrochloric acid, adding a slight excess of acid. Pass hydrogen sulfide into the hot solution until it is cold. Filter out the copper sulfide, observing the proper precautions to prevent its oxidation, and wash with 3 per cent ammonium chloride solution saturated with hydrogen sulfide. Dissolve the copper sulfide on the filter paper by washing with hot nitric acid (1 + 3) and wash with hot water. Evaporate the solution to dryness. Add a small quantity of water, transfer to a 50 cc. graduated flask, and make to volume.

Place 5-20 drops of potassium ferrocyanide (4 grams per 100 cc.), according to the quantity of copper present in the solution, in a tall clear glass cylinder or Nessler tube of 150 cc. capacity, add 5 cc. of ammonium nitrate solution (10 grams per 100 cc.) and then the whole or an aliquot portion of the above solution. Dilute this mixture to 150 cc. Place the same quantity of ferrocyanide and ammonium nitrate solutions in a second comparison tube, side by side with the one containing the sample, on a white tile or sheet of white paper. Run the standard copper solution (0.393 gram $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per liter 1 cc. = 0.0001 gram of Cu) into the comparison cylinder, stirring during the addition, until the color matches that of the sample. The number of cc. required multiplied by 0.0001 gives the weight of copper in the sample contained in the adjacent cylinder.

ZINC.

Boil the filtrate containing the zinc to expel the hydrogen sulfide. Make the solution decidedly ammoniacal and then decidedly acid with formic acid (1 + 1). Filter while hot to remove any insoluble matter and then pass in a stream of hydrogen sulfide for 15-20 minutes. Warm the solution 15 minutes on a steam bath; then remove for 30 minutes before filtration. Filter either on a filter paper or tared Gooch crucible, wash the precipitate of zinc sulfide with 2 per cent ammonium thiocyanate solution, dry, ignite, and weigh the zinc oxide.

Collaborative results.

(Expressed as parts per million.)

COLLABORATOR	SAMPLE A				SAMPLE B			
	METHODS				METHODS			
	TENTATIVE		PROPOSED		TENTATIVE		PROPOSED	
	Copper	Zinc	Copper	Zinc	Copper	Zinc	Copper	Zinc
R. M. Mehurin	76.0	282	68	308	8	96	14	104
	64.0	288	64	298	16	84	12	112
Leslie Hart	70.0	282	64	256	24	96	24	90
	82.0	266	48	280	35	86	24	96
E. H. Berry	30.5	299	42	292	11	109	24	95
	31.7		43		12		18	105

The proposed method was similar to the one studied in 1926 except for some slight changes in the details.

In addition, Mehurin determined the copper and zinc in the two samples, following details worked out by him. The results obtained, expressed as parts per million, were as follows:

SAMPLE A		SAMPLE B	
Copper	Zinc	Copper	Zinc
80	314	22	112
80	308	22	100

COMMENTS BY COLLABORATORS.

R. M. Mehurin.—The low copper results obtained by use of the tentative and proposed methods are, in my opinion, explained chiefly by the difficulty often encountered in the tentative method in obtaining a satisfactory end point when titrating very small quantities of copper with sodium thiosulfate, and in the proposed method by the difficulty encountered in dissolving the copper sulfide on the filter paper by means of a reasonable quantity of hot one to three nitric acid. The writer recovered relatively large quantities of copper from the filter paper by following up the hot one to three nitric acid treatment with hot one to one nitric acid. Even this did not recover all copper in case of Sample A, and the remainder was recovered upon ashing the paper in the muffle furnace at a dull red heat, as described in the writer's method¹.

When the large bulk of copper nitrate solution was evaporated to dryness in a porcelain dish, and the residue was taken up with water, it was found that the solution was turbid and required filtering. Also, a small quantity of copper had combined in some manner with the residue so as to render it insoluble in water. Upon the addition of a few drops of nitric acid, however, it readily went into solution.

The method of adding the standard copper solution to the second comparison tube is open to the objection that previous dilution of this tube is not prescribed and consequently the resulting concentration of copper ferrocyanide causes turbidity and a different shade of color from that in the tube with which it is to be compared, making accurate comparisons difficult. Furthermore, the necessarily slow additions of the standard copper solution to the second tube and the repeated comparisons necessary permit so much time to elapse that the shade of color of the first tube has changed materially before the final comparison is made. Where a series of standard tubes is used, as in the writer's method, all the solutions, both standard and sample, are diluted to mark first, and then the necessary potassium ferrocyanide is added on top of the diluted solution. In this way the formation of any appreciable quantity of copper ferrocyanide is prevented until the tubes are inverted and the solutions are mixed. In such cases, clear solutions of uniform color always result.

In regard to the proposed tentative method, needless to say I have found it very much more time-consuming, due chiefly to filtrations of large volumes of solutions, and not nearly so dependable in respect to the copper determination as my own method.

I have no special comment to make in regard to the two methods for zinc determination. There is no material difference between them, and both give satisfactory results.

Leslie Hart.—I much prefer the titration method for copper to the colorimetric method, as the sample and standard show different shades in the latter method, making comparison more or less a guess, as will be evidenced by the wide differences obtained. It seems probable that the titration method for copper would be applicable to the ashing method as well as the hydrolysis method. The proposed method is much less tedious than the tentative method.

¹ *J. Ind. Eng. Chem.*, 1923, 15: 942.

From a study of the results it will be seen that those for copper are very unsatisfactory. As the results obtained by Mehurin using his details were reasonably satisfactory, it would seem that these details should be tried out next year. The method resembles that of the proposed method, varying essentially in the details.

The results obtained on zinc were fairly satisfactory.

The preparation of the sample by ashing has proved very satisfactory and certainly is much to be preferred to the acid hydrolysis method.

RECOMMENDATIONS¹.

It is recommended that the study of the preparation of the sample by ashing be continued another year and that the details as outlined by Mehurin be studied, as well as such other methods as the referee may be able to develop.

No report on spices and other condiments was given by the associate referee.

REPORT ON CACAO PRODUCTS.

By H. A. LEPPER (Food, Drug and Insecticide Administration, Washington, D. C.), *Referee*.

The work this year was limited, resulting solely in a report by Marie L. Offutt, Associate Referee on Crude Fiber in Cacao Products, of progress in the development of a method for determining crude fiber.

It is recommended¹—

(1) That the method for crude fiber proposed by the associate referee be further studied collaboratively and that the studies be extended to milk cacao products.

(2) That methods for the determination of milk solids and sucrose in cacao products be studied.

(3) That study be continued on methods for the detection of foreign fats in cacao fat and in the fat of cacao products in general, with special attention to quantitative aspects.

REPORT ON CRUDE FIBER IN CACAO PRODUCTS.

By MARIE L. OFFUTT (Food, Drug and Insecticide Administration, New York, N. Y.), *Associate Referee*.

The collaborative study of the tentative and proposed methods suggested in last year's report was carried out on two samples—a bitter

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 84.

chocolate and a sweet chocolate made from the bitter. The following directions were sent with the two samples to collaborators:

CRUDE FIBER—(PROPOSED METHOD).

Weight of sample; weight of ether, alcohol, water, insoluble material; per cent crude fiber.

CRUDE FIBER—(TENTATIVE METHOD).

(Results to be expressed in percentage.)

Moisture, fat, sucrose, crude fiber, crude fiber (calculated to water-, fat- and sugar-free basis).

The following methods should be used:

- (1) *Moisture*: Proceed as directed on p. 115, 2, *Methods of Analysis*, A. O. A. C., 1925.
- (2) *Fat*: Lepper and Waterman method. *Journal*, A. O. A. C., Vol. VIII, No. 6, p. 706. (Use this fat-free sample for crude fiber determination.)
- (3) *Sucrose*: Proceed as directed on p. 344, 13.
- (4) *Crude fiber*: Proceed as directed on p. 118, 17, observing usual precautions. Calculate this fiber figure to moisture-free, fat-free basis for liquor and moisture-free, fat-free, sugar-free basis for sweet chocolate.

(5) *Crude fiber* (Proposed method):

Treat 15 grams of liquor or 50 grams of sweet chocolate with 100 cc. of ether in a nursing bottle, centrifuge, and decant the supernatant liquor twice; dry the residue in an oven at about 100°C. and then powder in the bottle with a flattened glass rod. (In some cases it may be found necessary to grind the material in a mortar and extract a third time with ether.) Wash in the nursing bottle with three 100 cc. portions of distilled water at room temperature, shaking well each time until no cocoa material adheres to the bottle. Centrifuge after each washing 10–15 minutes and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc. portions of 95 per cent alcohol and one 100 cc. portion of ethyl ether. Transfer the residue to a platinum dish, dry to constant weight, and grind in a mortar. Weigh 2 grams of the dried material and determine crude fiber by the usual A. O. A. C. method. Report results as the percentages of crude fiber in the washed and dried material.

The chemists reporting and to whom acknowledgment is made, are—

S. C. Rowe, Food, Drug and Insecticide Administration, Washington, D. C.
 H. I. Macomber, Food, Drug and Insecticide Administration, New York, N. Y.
 W. C. Taber, Food, Drug and Insecticide Administration, San Francisco, Calif.
 W. T. Mathis, Food, Drug and Insecticide Administration, New Haven, Conn.
 Louis G. Petree, Food, Drug and Insecticide Administration, Seattle, Wash.

The results of collaborators are summarized in Table 1. The figures obtained by the proposed method are somewhat higher than those obtained by the tentative method, as would be expected, since the washings with alcohol and water remove an appreciable amount of the cacao material. The variation in the results obtained by analysts using the proposed and tentative methods on the bitter chocolate is about the same, but in the case of the sweet chocolate the variation noted when the tentative method was used on the basis on which reported is twice that found by the proposed method. The results for crude fiber determined by the tentative method on original basis differ by only 0.43, but

on the calculated basis the difference becomes 3.26, due to the factor used in changing over. It would seem that a method that reports on the material direct would be a step closer to the true value. However, more collaborative work should be done on the proposed method before any definite conclusion is drawn.

COMMENTS BY COLLABORATORS.

S. C. Rowe suggested the use of 70 per cent alcohol instead of water for washings as a certain amount of chocolate particles floats in the water washings. He also mentioned the Lepper and Waterman method for milk protein extraction¹ as a possibility in work on milk chocolate fiber content.

W. C. Taber also suggested an alcohol and water mixture for washings.

A few results by both the tentative and proposed methods were given in last year's report². Table 2 gives the results by both methods on 26 more samples. The proposed method seems to give very good checks, and when the tentative gives decidedly high results, the proposed is also higher.

Table 3 gives results obtained by the proposed method on some known samples of liquor and sweet chocolates made from these liquors, and also on the milk chocolates of these liquors. A proposed method for milk chocolate, which will be discussed further, was used. Here again the agreement of results for liquor and its sweet chocolate and also of those obtained by the new proposed method for milk chocolate fiber seems close.

The method proposed in last year's report was not applicable to milk chocolate, so further work was done along that line. A modification of the proposed method was tried by increasing the number of water washings. Results were too low on the known sample used. Sodium oxalate and 2 per cent and 1 per cent sodium tetraborate were then used, followed by three washings with water, but the results continued low. The difficulty seemed to lie in washing out all the milk solids. Finally, a modification of the Lepper and Waterman method for the determination of milk protein in cacao products was used; it gave very satisfactory results, as noted in Table 3. The method used is as follows:

PROPOSED METHOD.

SPECIAL REAGENTS.

(a) *Acidified alcohol*.—Add 10 cc. of glacial acetic acid to 895 cc. of 95 per cent alcohol and make up the volume to 1 liter with distilled water.

(b) *Weakly acid aqueous medium*.—Dilute concentrated hydrochloric acid to about 100 times its volume with distilled water to get roughly a 0.1 *N* solution. Titrate and prepare 0.015 *N* hydrochloric acid by making calculated dilution. Add 5 grams of anhydrous sodium sulfate per liter to this solution.

¹ *J. Ind. Eng. Chem.*, 1927, 19: 501.

² *This Journal*, 1928, 11: 515.

TABLE 1.

Collaborative results.

BITTER CHOCOLATE (15 GRAM SAMPLE).

COLLABORATOR	PROPOSED METHOD		TENTATIVE METHOD				
	Weight of Ether, Alcohol, Water, Insoluble Matter	Crude Fiber	Moisture	Fat	Crude Fiber	Sucrose	Crude Fiber Calculated to Moisture, Fat, Sugar-free Basis
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
S. C. Rowe	4.6892	10.19	1.82	52.20	3.18	6.93
	4.6682	10.13	1.90	52.32	3.25	7.08
	Av. 4.6787	Av. 10.16	Av. 1.86	Av. 52.26	Av. 3.22	Av. 7.01
H. I. Macomber	4.9510	8.60	1.57	52.08	3.39	7.32
	5.1183	8.98	1.62	52.04	3.36	7.25
	Av. 5.0347	Av. 8.79	Av. 1.60	Av. 52.06	Av. 3.38	Av. 7.29
W. C. Taber	8.85	2.13	52.60	3.03	6.69
	8.88	2.13	3.05	6.73
	Av. 8.87	Av. 2.13	Av. 3.04	Av. 6.71
M. L. Offutt	4.8447	9.22	1.68	52.21	3.18	6.91
	4.8409	9.08	1.82	52.26	3.17	6.89
	4.8509	9.07					
W. T. Mathis	Av. 4.8455	Av. 9.12	Av. 1.75	Av. 52.24	Av. 3.18	Av. 6.91
	4.7732	8.94	1.98	51.93	2.83	6.23
	4.7530	9.11	2.02	51.89	2.85	6.28
Louis G. Petree	Av. 4.7631	Av. 9.03	Av. 2.00	Av. 51.91	Av. 2.84	Av. 6.26
	4.6920	9.74	2.00	52.16	3.14	6.88
	4.7149	9.51	2.00	52.36	3.18	6.94
Maximum	Av. 4.7035	Av. 9.63	Av. 2.00	Av. 52.26	Av. 3.16	Av. 6.91
		10.16					7.29
		8.79					6.26
Minimum		9.43					6.85
		1.37					1.03
Average							
Variation							

(c) *Sodium tetraborate*.—Prepare a 0.50 *M* solution (31.012 grams per liter) of the purest obtainable dried boric acid. (Dry the acid over calcium chloride to constant weight since heat causes boric acid to lose water of constitution even below 50°C.) To 104.6 cc. of the 0.50 *M* boric acid add 62.15 cc. of exactly 1 *N* carbonate-free sodium hydroxide (or an exactly equivalent quantity of any convenient normality) and make up the volume with recently boiled and cooled distilled water to 1 liter.

DETERMINATION.

Treat 50 grams of milk chocolate with two 100 cc. portions of ether in a nursing bottle, centrifuge and decant the supernatant liquor, dry the residue in an oven at

TABLE 1—Continued.

Collaborative results.

SWEET CHOCOLATE (50 GRAM SAMPLE).

COLLABORATOR	PROPOSED METHOD		TENTATIVE METHOD				
	Weight of Ether, Alcohol, Water, Insoluble Matter	Crude Fiber	Moisture	Fat	Crude Fiber	Sucrose	Crude Fiber Calculated to Moisture- & Fat-, Sugar-free Basis
	<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
S. C. Rowe	4.7780	10.55	0.63	32.92	1.16	51.66	7.86
	4.7501	10.73	0.62	32.88	1.13	51.77	7.66
	Av. 4.7640	Av. 10.64	Av. 0.63	Av. 32.90	Av. 1.15	Av. 51.72	Av. 7.76
H. I. Macomber	5.0524	10.03	0.53	33.23	1.35	52.09	9.70
	5.0728	10.21	0.56	33.10	1.29	52.62	9.27
	Av. 5.0626	Av. 10.12	Av. 0.55	Av. 33.17	Av. 1.32	Av. 52.36	Av. 9.49
W. C. Taber	9.16	0.63	33.03	1.15	52.48	8.30
	9.08	0.67	33.09	1.4	7.56
	Av. 9.12	Av. 0.65	Av. 33.06	Av. 1.10	Av. 7.93
M. L. Offutt	4.6549	9.76	0.64	33.08	1.07	51.15	7.12
	4.8324	9.37	0.59	33.10	1.09	51.47	7.26
	4.8219	9.60		32.97	1.06		7.06
	Av. 4.8364	Av. 9.58	Av. 0.62	Av. 33.05	Av. 1.07	Av. 51.31	Av. 7.15
W. T. Mathis	4.9705	9.30	0.92	32.86	0.95	51.88	6.64
	4.9870	9.68	0.98	32.88	0.83	51.88	5.81
	Av. 4.9788	Av. 9.49	Av. 0.95	Av. 32.87	Av. 0.89	Av. 51.88	Av. 6.33
Louis G. Petree	4.6895	10.22	0.74	32.66	1.07	50.70	6.94
	4.6523	10.25	0.70	32.80	1.09	51.50	7.07
	Av. 4.6709	Av. 10.24	Av. 0.72	Av. 32.73	Av. 1.08	Av. 51.10	Av. 7.00
Maximum		10.64					9.49
Minimum		9.12					6.23
Average		9.87					7.59
Variation		1.52					3.26

about 100°C., and powder the residue in the bottle with a flattened glass rod. Wash in a centrifuge bottle with two 100 cc. portions of acidified alcohol, shaking each time until no material adheres to the sides of the bottle, centrifuge and decant each portion, shake next with 100 cc. weakly acid aqueous medium, centrifuge and decant. Then wash with two 100 cc. portions of 95 per cent alcohol and one portion of 100 cc. of ethyl ether. Dry at 100°C., powder as before, and wash with one 100 cc. portion of sodium tetraborate, centrifuge, and decant. Wash three times with 100 cc. of water followed by two 100 cc. of 95 per cent alcohol and one 100 cc. of ether. Dry at 100°C. to constant weight in a platinum dish, grind in a mortar, and weigh out 2 grams of dried material. Determine the crude fiber by the usual A. O. A. C. method and report results as the fiber in the washed and dried material.

TABLE 2.
Collaborative results on crude fiber determined by tentative
and proposed methods.

SAMPLE	TENTATIVE METHOD*	PROPOSED METHOD			
	per cent	per cent			
434	8.30	9.06,	9.17	Av.	9.12
447	7.21	8.78,	9.05	"	8.92
879	6.77	9.12,	9.22	"	9.17
880	6.27	8.34,	8.12	"	8.23
901	5.83	8.29,	8.57	"	8.43
902	6.00	8.31,	8.66	"	8.49
256	7.35	9.08,	8.86	"	8.97
257	6.60	8.81,	8.79	"	8.80
950	6.66	8.89,	8.70	"	8.80
654	6.79	8.54,	8.58	"	8.56
70	7.03	8.11,	8.13	"	8.12
91	7.06	8.69,	8.61	"	8.65
803	5.23	8.25,	8.12	"	8.19
804	6.31	8.53,	8.57	"	8.55
053	7.19	8.93,	9.00	"	8.97
054	6.22	8.54,	8.54	"	8.54
72	6.64	9.24,	9.29	"	9.27
61	9.33	10.19,	10.30	"	10.25
58	6.29	9.52,	9.65	"	9.59
60	8.85	10.23,	10.14	"	10.19
904	9.13	9.80,	9.73	"	9.77
A	6.77	8.41,	8.54	"	8.48
B	7.85	8.50,	8.22	"	8.36
C	7.77	9.02,	8.79	"	8.91
D	8.25	9.25,	9.22	"	9.24
E	7.00	8.11,	7.76	"	7.94

* Results are on moisture-, fat-, and sugar-free basis.

TABLE 3.
Collaborative results on crude fiber determined by proposed
and milk chocolate proposed methods.

SAMPLE	PROPOSED METHOD			MILK CHOCOLATE PROPOSED METHOD		
A	8.41,	8.54	Av. 8.48			
AS	8.59,	8.75	" 8.67			
AM				8.77,	8.70	Av. 8.74
AMB				8.71,	8.41	" 8.56
B	8.50,	8.22	" 8.36			
BS	8.63,	8.81	" 8.72			
BM				8.67,	8.57	" 8.62
C	9.02,	8.79	" 8.91			
CS	9.70,	9.43	" 9.57			
CSF	9.05,	9.11	" 9.08			
CM				8.92,	8.88	" 8.90
CMF				9.39,	9.63	" 9.51
D	9.25,	9.22	" 9.24			
DS	9.87,	9.56	" 9.72			
DSF	9.15,	9.08	" 9.12			
DM				9.58,	9.76	" 9.62
E	7.76,	8.11	" 7.94			
F	8.09,	7.70	" 7.90			
H	7.99,	7.84	" 7.92			
G				7.39,	7.70	" 7.55

NOTE:—

A, B, C, D, E = bitter liquors.

S after above letters means sweet chocolate made from same.

F, H also sweet chocolates from E.

SF = sweet chocolate refined further.

M = milk chocolate.

MF = milk chocolate refined.

In Table 4 is given the crude fiber determined by the proposed method (Table 1), when calculated to the moisture-, fat-, sugar-free basis. The results of the various analysts agree very closely, and the variation is cut from 1.03 to 0.69 on the bitter and from 3.26 to 1.19 on the sweet chocolate. The agreement between the fiber of the bitter and of the sweet chocolate is also closer than when it is determined by the tentative method.

TABLE 4.

*Percentage of crude fiber on moisture-, fat-,
sugar-free basis by proposed method.*

ANALYST	CHOCOLATE	
	BITTER	SWEET
S. C. Rowe.....	6.91	6.86
H. I. Macomber.....	6.37	7.36
M. L. Offutt.....	6.40	6.17
W. T. Mathis.....	6.22	6.61
L. G. Petree.....	6.60	6.19
Maximum.....	6.91	7.36
Minimum.....	6.22	6.17
Average.....	6.50	6.64
Variation.....	0.69	1.19

RECOMMENDATION¹.

It is recommended that further collaborative work be done on the proposed methods for the determination of crude fiber in bitter, sweet and milk chocolates.

No report on cacao butter was given by the associate referee.

No report on naval stores was given by the associate referee.

REPORT ON TURPENTINE.

By V. E. GROTLISCH (Food, Drug and Insecticide Administration,
Washington, D. C.), *Associate Referee*.

At the 1925 meeting of the association several modifications were made in the tentative alternative (sulfuric-fuming nitric acid) method for the determination of mineral oil in turpentine with the view to simplification and greater rapidity of operation. This method has continued to receive attention, not with the idea of supplanting the present official method, which specifies the use of a carefully standardized fuming sulfuric acid, but of providing another accurate method for use in labora-

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 84.

tories where only an occasional analysis of turpentine is made, and the expenditure of time and work in making up the standardized 38 times normal fuming sulfuric acid is not warranted.

No reports were made at the 1926 and 1927 meetings of the association, primarily because laboratory studies conducted by the associate referee had led to the belief that an even simpler method, giving reliable results without resorting to the use of fuming nitric acid, could be presented for collaborative study. However, when it seemed impossible to develop such a method the present tentative method was again taken up for study. The fairly uniform results obtained indicate that it can be recommended as an official method of the association when more data concerning the variation in the physical constants of the residues from pure and adulterated samples are available.

During the last year the experiments conducted in the Naval Stores Laboratory indicated that the steam distillation of the sulfuric acid-terpene polymerization mixture could be further shortened without affecting results, since no traces of mineral oil could be found in the distillate after 300 cc. had been collected. Any oily distillate collected thereafter contained only terpene compounds. Consequently, the instructions sent out to the collaborators this year embodied this modification. Additional changes made consisted of (1) a reduction in the quantity of sample used, (2) elimination of the preliminary steam distillation, (3) separation of the fuming nitric acid from the oil, after nitration, before the rather energetic reaction which sets in when the mixture warms to room temperature, as this was found to lead to low results owing to partial destruction of the mineral oil.

The instructions which were sent out are the following:

Place 50 cc. of the turpentine in a 300 cc. Kjeldahl or other long-necked flask; cool in ice water; and add slowly, with constant agitation, 25 cc. of concentrated sulfuric acid. Shake well to obtain complete reaction, keeping the flask cool. When the reaction is complete, cool thoroughly and add 25 cc. of water. Distil the polymerized mixture in a current of steam, collecting 300 cc. of total distillate. Separate the oil from the aqueous portions.

Place a volume of fuming nitric acid (sp. gr. 1.5) equal to three times the volume of the oil in a 200-250 cc. separatory funnel, and cool in ice water. Add the oil cautiously drop by drop, shaking carefully and keeping the mixture cool. Allow the funnel to stand quietly, very lightly stoppered, until the oil comes to the surface (about 30 seconds). Then draw off the acid and wash the remaining oil well with a little fuming nitric acid, once with strong nitric acid, and finally several times with water. Measure the volume of the oil, record its consistency and color, and determine the refractive index at 20°C. Pure gum spirits of turpentine gives less than 0.5 per cent residue by this method.

Two samples were sent out for study, one consisting of a pure gum turpentine and the other of gum turpentine with 10 per cent of a mineral

TABLE 1.
Collaborative results.

SAMPLE 1.

ANALYST	QUANTITY OF RESIDUE		REFRACTIVE INDEX AT 20°C.	COLOR	CONSISTENCY
	<i>cc.</i>	<i>per cent</i>			
1	0.3	0.6	1.4552	Deep yellow	Oily Limpid
	0.2	0.4	1.4500		
2	0.2	0.4	1.4541	Light gold yellow	Limpid
3	0.15	0.3	Straw yellow	Light oil
4	0.25	0.5	1.492	Slightly yellowish	Thin
	0.35	0.7	1.489		
5	Quantity of residue too small to permit observations				
6	0.1	0.2	Dark straw	
	0.2	0.4			
7	0.25	0.5	1.4450	Light straw	Limpid
8	0.6	1.2	1.4596	Yellow	
	0.51	1.02	1.4513		

SAMPLE 2.

1	5.2 4.8	10.4 9.6	1.4312 1.4285	Pale straw	Limpid
2	4.6	9.2	1.4298	Almost colorless	Limpid
3	4.2	8.4	1.4275	Almost colorless	Limpid like kerosene
4	6.30	12.6	1.444	White	Thin
	6.20	12.4	1.442		
5	5.2	10.4	1.4390	Pale yellow	Thin
	5.1	10.2			
6	3.2	6.4	1.4210	Practically colorless	Limpid
	3.2	6.4	1.4205		
7	4.9	9.8	1.4272	Colorless	Limpid
8	3.88	7.76	1.4300	Pale yellow	
	3.98	7.96	1.4270		

oil paint thinner. The analytical results are shown in the table. The following collaborators, referred to by number in the table, reported:

1. V. E. Grotlisch.
2. T. O. Pappe, Food, Drug and Insecticide Administration, Baltimore, Md.
3. F. L. Elliott, Food, Drug and Insecticide Administration, Baltimore, Md.

4. L. A. Salinger, Food, Drug and Insecticide Administration, Savannah, Ga.
5. H. Solomon, A. & M. College, Miss.
6. Leslie Hart, Food, Drug and Insecticide Administration, Chicago, Ill.
7. W. C. Smith, Bureau of Chemistry and Soils, Washington, D. C.
8. H. R. Salzberg, Mellon Institute, Pittsburgh, Pa.

When it is considered that the collaborating analysts, with the exception of the referee and W. C. Smith, had had no previous experience with this method of analyzing turpentine, the results shown in the table are quite encouraging. In the case of Sample No. 1, a pure turpentine, only one analyst reported a recovery of more than 0.7 per cent residue after nitration, while most of the collaborators got not more than 0.5 per cent. Furthermore, in only one case was the refractive index of this residue reported as lower than 1.4500 at 20°C. In the 1925 work the only refractive index reported on this residue from pure turpentine was 1.4580.

On Sample No. 2, containing 10 per cent mineral oil, the results reported by several analysts showed practically quantitative recovery of the adulterant. The average of all the reports was 9.3 per cent. The results do not vary quite so much from theoretical as those obtained when the official method was studied and adopted in 1924.

RECOMMENDATION¹.

It is recommended that the tentative sulfuric-fuming nitric acid method for determining mineral oil in turpentine, as modified, be further studied, with particular reference to the means of distinguishing between the normal residue from pure turpentine and that from a slightly adulterated turpentine.

No report on paints, paint materials, and varnishes was given by the referee.

The proceedings for Monday were published in Vol. XII, No. 1.

¹ For report of Subcommittee C and action of the association, see *This Journal*, 1929, 12: 73.

CONTRIBUTED PAPERS.

DETERMINATION OF NITRATES AND NITRITES IN WHEY.

By ERIK OHLSSON and HUGO FREDHOLM (Laboratory of Chemistry,
University of Lund, Sweden).

INTRODUCTION.

On many occasions it has been found desirable to determine nitrates and nitrites when they occur together and in the presence of a large quantity of organic matter. The writers tried to work out a method that might be of interest to toxicologists in nitrite poisoning cases as well as to dairy, agricultural and food chemists. The procedure consists in extracting nitric acid with ether in Widmark's¹ apparatus before reducing it to ammonia and making the determination. In the presence of nitrites the sum of the nitrates and nitrites is determined in one aliquot after the nitrous acid is oxidized to nitric acid with potassium permanganate, while the nitrates are determined in another aliquot after the nitrites have been destroyed by 2,4-diamino-6-oxy-pyrimidine.

REDUCTION OF NITRATES TO AMMONIA.

The reduction of nitrates to ammonia can be accomplished in several ways. The writers have used the method given by Scales and Harrison², which seems particularly easy and as accurate as any other. It consists in reducing nitric acid by coppered zinc in the presence of magnesia, distilling the ammonia formed into boric acid, and titrating with hydrochloric acid with bromphenol blue as indicator.

The titration requires some care to obtain true values. A blank determination must be made with the same reagents. A series of such determinations gave values between 0.30 and 0.37 cc. of 0.1 *N* hydrochloric acid. The titer of the hydrochloric acid is best determined with ammonium chloride in the same manner as in the determination of nitrates.

The accuracy of the method is shown by the results given in Table 1. The largest error is 0.007 millimols, or 0.07 cc. of 0.1 *N* hydrochloric acid.

NITRATES IN ABSENCE OF NITRITES.

In aqueous solutions.—Pour the solution to be extracted—not more than 50 cc.—into one half of Widmark's apparatus and add 10 cc. of 0.1 *N* sulfuric acid. In the other half of the apparatus introduce 20 cc. of 0.5 *N* sodium hydroxide, and fill the vessel with a suitable quantity of ether (in the work following 400 cc. was used). Then carry out the extraction as described by Widmark.

When the extraction is completed, draw off the sodium hydroxide solution and wash this part of the apparatus three times with approximately 10 cc. of water. Before the

¹ *Skand. Arch. Physiol.*, 1926, 48: 61; *Bull. soc. chim. biol.*, 1928, 10: 669.

² *J. Biol. Chem.*, 1916, 27: 327; 1921, 46: 53; *J. Ind. Eng. Chem.*, 1920, 12: 350.

TABLE 1.
Determination of aqueous solutions of potassium nitrate by the Scales-Harrison method.

CALCULATED	FOUND
<i>millimols</i>	<i>millimols</i>
1.309	1.310
1.023	1.030
1.007	1.013
1.007	1.012
1.007	1.011
1.007	1.011
1.007	1.009
1.007	1.005
1.007	1.004
0.917	0.916
0.896	0.890
0.506	0.505
0.506	0.503
0.506	0.503

nitric acid is determined in the mixture of sodium hydroxide and wash-water, as described by Scales and Harrison, remove the accompanying ether by warming it on the water bath; then cool and neutralize with sulfuric acid.

In these extractions the time of rocking to and fro was 26 seconds and the angle was 18° to each side. Since nitric acid is less soluble in ether than in water, the time of extraction should be rather long. To determine the time required for complete extraction, a series of extractions was made under constant conditions but with varying periods of time. The results are shown in Table 2. These experiments show that half of the nitric acid is extracted in about 4 hours. In 48 hours more than 99.9 per cent would be extracted, providing the quantity extracted

TABLE 2.
Extraction of 40 cc. of a solution of potassium nitrate containing 1.007 millimols.

TIME OF EXTRACTION	QUANTITY EXTRACTED	EXTRACTION-COEFFICIENT
<i>hours</i>	<i>millimols</i>	
1	0.147	0.07
2	0.342	0.09
3	0.404	0.07
4	0.498	0.07
8	0.767	0.08
15	0.967	0.09
23	0.996	0.09
42	1.011

in a unit of time is proportional to the concentration, at any moment, of the unextracted nitric acid. The experiments show that this is at least approximate. The extraction coefficient was calculated from this according to the law of a monomolecular reaction.

In the presence of precipitated protein or other sediments, however, the extraction is probably a little slower; therefore, in the work here described the writers always extracted for from 60 to 70 hours¹. For practical use, however, an extraction period of 48 hours should be sufficient in such cases. Even after a 24 hour extraction period there should remain only a small percentage of nitric acid.

The reliability of the method is shown in Table 3, where the results of extracting a known quantity of nitrate are given.

TABLE 3.
*Extraction of 40 cc. of an aqueous solution
of potassium nitrate.*

CALCULATED	FOUND
<i>millimols</i>	<i>millimols</i>
1.282	1.271
1.007	1.004
0.804	0.808
0.649	0.655
0.506	0.506
0.327	0.326
0.206	0.208
0.000	0.002

In whey.—The determination of nitrates in whey is made in the same manner as in aqueous solutions and offers no difficulties. When the sulfuric acid is added there is formed a precipitate of protein, which partly sinks to the bottom of the apparatus. It is advisable in the course of the extraction to stir up this precipitate once or twice with a glass rod. Table 4 gives results of determinations of known quantities of potassium nitrate in a mixture of 10 cc. of water and 30 cc. of whey, which were extracted as indicated. They show that the method is applicable to the analysis of whey.

TABLE 4.
Determination of nitrates in whey.

CALCULATED	FOUND
<i>millimols</i>	<i>millimols</i>
1.007	1.005
0.506	0.505
0.506	0.503
0.417	0.413
0.206	0.208
0.206	0.206
0.000	0.000

¹It is assumed that this period of extraction was used in obtaining results reported in Tables 1 to 6, inclusive.—*Editor*.

NITRATES IN THE PRESENCE OF NITRITES.

When nitrates are to be determined in the presence of nitrites, the latter must first be destroyed. Methods of accomplishing this in an acid reaction are faulty because the nitrous acid that is formed is partly decomposed into nitric acid and oxides of nitrogen before the required reaction is completed. However, Hahn¹ has suggested another method which can be carried out in a neutral solution and which consequently does not have this disadvantage. The method consists in adding 2.4-diamino-6-oxy-pyrimidine sulfate to the solution containing nitrites. In a short time 2.4-diamino-5-nitroso-6-oxy-pyrimidine, which is slightly soluble, is formed. This method has proved to be particularly suitable for the authors' purpose.

NOTE.—The compound 2.4-diamino-6-oxy-pyrimidine is made according to Traube², in the following manner: Dissolve 5 grams of sodium in 100 cc. of absolute alcohol and add a solution of 10 grams of guanidine hydrochloride in 100 cc. of absolute alcohol. Do not filter off the precipitate of sodium chloride but add at once 12 grams of cyanoacetic acid. Introduce the mixture into a flask, connect with a reflux condenser, and boil on the water bath for 30 minutes. After cooling, decant the alcoholic solution and evaporate to dryness on the water bath. Dissolve this residue in water and mix with the residue in the flask. Acidify the solution thus obtained with dilute sulfuric acid, whereupon 2.4-diamino-6-oxy-pyrimidine sulfate precipitates as a pale-yellow colored crystalline powder, which is filtered with suction, washed three times with water, and dried. This product can be used without purification. The yield obtained was about 18 grams, or 85 per cent of the theoretical.

Aqueous solutions of nitrates and nitrites.—Add 0.5 grams of 2.4-diamino-6-oxy-pyrimidine sulfate to known quantities of nitrate and nitrite dissolved in water. (The red-colored 2.4-diamino-5-nitroso-6-oxy-pyrimidine separates almost immediately.)

TABLE 5.

Determination of nitrates in aqueous solutions and in whey in the presence of nitrites.

POTASSIUM NITRATE	SODIUM NITRITE	NITRATE FOUND
<i>millimols</i>	<i>millimols</i>	<i>millimols</i>
IN AQUEOUS SOLUTIONS		
1.007	1.61	1.013
1.007	1.51	1.022
1.007	0.00	1.003
0.502	1.84	0.500
0.502	0.61	0.498
0.204	0.91	0.202
0.204	0.72	0.204
0.000	1.45	0.011
IN WHEY		
1.007	0.97	1.008
1.002	0.73	1.000
0.506	0.78	0.508
0.502	0.59	0.498
0.204	1.09	0.206
0.204	0.73	0.202
0.000	1.15	0.010
0.000	0.62	0.005

¹ Ber., 1917, 50: 705.

² Ibid., 1900, 33: 1871; 1913, 46: 3839.

Allow the mixture to stand for 30 minutes, stirring once or twice. Then introduce the mixture into the extracting apparatus without filtering, add sulfuric acid, and make the extraction in the usual manner.

Whey containing nitrates and nitrites.—Make the determinations exactly as for the analysis of aqueous solutions of these two compounds.

Table 5 shows the results of such a series when known quantities were determined.

SUM OF NITRATES AND NITRITES.

Aqueous solutions.—This method is based upon the well-known fact that nitrous acid is very easily oxidized to nitric acid by potassium permanganate.

The experiments were made in the following manner:

To 30 cc. of a solution containing known quantities of nitrate and nitrite add 5 cc. of a 5 per cent solution of potassium permanganate; then add 10 cc. of 0.1 *N* sulfuric acid quite slowly, drop by drop, so that the solution may not become too acid before the nitrous acid is oxidized. After several minutes add a few drops of a saturated solution of oxalic acid to destroy the excess of the oxidizing reagent. Then extract the solution in the usual manner. This series of experiments shows no greater errors than analyses of solutions containing nitrates only.

TABLE 6.

Determination of the sum of nitrates and nitrites in aqueous solutions and in whey.

POTASSIUM NITRATE	SODIUM NITRITE	SUM OF NITRATE AND NITRITE	
		Calculated	Found
<i>millimols</i>	<i>millimols</i>	<i>millimols</i>	<i>millimols</i>
IN AQUEOUS SOLUTIONS			
0.000	1.112	1.112	1.109
0.000	1.112	1.112	1.109
0.506	0.559	1.065	1.072
0.206	0.559	0.765	0.763
0.000	0.559	0.559	0.552
0.000	0.559	0.559	0.552
0.506	0.227	0.733	0.726
0.206	0.227	0.433	0.440
0.000	0.227	0.227	0.225
1.007	0.000	1.007	1.002
IN WHEY			
0.206	1.112	1.318	1.229
0.000	1.112	1.112	1.104
0.506	0.559	1.065	1.070
0.206	0.559	0.765	0.771
0.000	0.559	0.559	0.556
1.007	0.227	1.234	1.224
0.206	0.227	0.433	0.437
0.000	0.227	0.227	0.232
0.506	0.000	0.506	0.509
0.000	0.000	0.000	0.002

Whey containing nitrates and nitrites.—These determinations are made in the same manner as those of the aqueous solutions, except that addition of oxalic acid as a rule is unnecessary, because the whey contains sufficient organic material to reduce the excess of permanganate. Nitrous acid is oxidized more readily, however, than the organic substances, so the latter do not interfere with the determination.

Table 6 shows a series of determinations made with varying quantities of potassium nitrate and sodium nitrite dissolved in whey. The errors of analysis are of the same order as those previously obtained, with the exception of the first determination, although in this instance it amounts to only 1.4 per cent.

ALDEHYDE-OXIDATION REACTIONS FOR PHENOLS, PARTICULARLY THE OPIUM ALKALOIDS.

By CHARLES C. FULTON (U. S. Prohibition Bureau, Omaha, Nebr.).

The color reactions of phenols with formaldehyde and other aldehydes in the presence of strong acid are well known. Apparently it has not been recognized heretofore that the addition of an oxidizing agent to this system may give an entirely new reaction. This "combination" reaction may appear to be an intensification of the phenol-aldehyde reaction, or of the phenol-oxidation reaction, or it may be entirely different from either. Such combination reactions have been reported in the past, but always, so far as the writer can discover, as particular cases, and never as examples of a general phenolic reaction. Every opium alkaloid that the writer has studied gives a true combination reaction.

The reaction seems to be essentially an oxidation of the phenol-aldehyde compound. The particular color produced depends more on the phenol than on the aldehyde, but even with a given phenol and a given aldehyde the test may change with the strength of the oxidizing agent, the proportion of aldehyde, or the strength of the acid. All these reactions in concentrated sulfuric acid succeed with various phenolic compounds, such as ethers of phenols, as well as with true phenols.

USES OF THE REACTION.

The reaction of a phenol-aldehyde-acid-oxidant mixture will be useful in a number of ways:

- (1) As a means of identifying the opium alkaloids.
- (2) As a means of identifying other phenols and phenolic compounds.

In the formaldehyde-oxidation reactions obtained by the writer with ordinary phenols, the color produced has usually been red, but with a

complex phenolic compound it may be any color; it cannot be foretold in advance of experiment.

(3) As a means of detecting and identifying formaldehyde. Some of the reactions are so sensitive that although they must take place in fairly strong acid, the test can be so made as to detect 1 part of formaldehyde in 500,000 or even 1,000,000 parts of aqueous solution. The identification is not so good, since some other aldehydes may give colors like those produced by formaldehyde. If, however, a distillate is used and the test is tried with several of the suitable alkaloids (pseudomorphine, apomorphine, codeine, and papaverine), the identification is fairly certain.

(4) As a means of identifying a substance as an aldehyde. For some reason pseudomorphine, as the phenol, seems to have the most general action. With concentrated sulfuric acid and the nitric acid oxidizing agent it is almost a general reagent for aldehydes, giving a green or blue color. Apomorphine has a fairly general action. In this identification it should be remembered that a non-aldehyde may be decomposed by concentrated sulfuric acid, one or more aldehydes being formed.

PREVIOUS WORK.

Various unrecognized aldehyde-oxidation tests for phenols are scattered through the literature. The reaction of pseudomorphine with Marquis' reagent has more than once been reported green instead of red, probably because the acid used contained oxidizing impurities. Similar mistakes have been made with other phenolic substances.

Descriptions of the "furfural test" for phenols are confusing. Sugar solution is generally used instead of furfural, and while sugar decomposes with concentrated sulfuric acid to give furfural, it also gives a trace of formaldehyde, and with a little oxidizing agent present it is likely to be the formaldehyde that produces the noticeable reaction.

Lloyd's test¹, in which a mixture of morphine and hydrastine is supposed to simulate the "fading purple" test of strychnine, is one in which the hydrastine plays the part of an aldehyde. Hydrastine, on hydrolysis, gives opianic acid, an aldehyde, and on hydrolytic oxidation it also gives hydrastinine, another aldehyde.

In 1914 L. Grimbart and A. Leclerc² published the following as an identification test for pseudomorphine (oxydimorphine, oxymorphine): "Pure oxymorphine, free from any trace of $K_3Fe(CN)_6$, turns reddish with formol- H_2SO_4 * * *, but a trace of powdered $K_3Fe(CN)_6$ turns this emerald-green".

¹ Peterson, Haines and Webster. *Legal Medicine and Toxicology* (W. B. Saunders Co., Phila.), Vol. II, p. 581 (1923). Fuller. *Chemistry and Analysis of Drugs and Medicines* (John Wiley & Sons, N. Y.), p. 190 (1920).

² *J. pharm. chim.*, 1914, 10: 425; *C. A.*, 9: 1224.

In 1915 Warren reported that when papaverine is intimately mixed with a little powdered potassium ferricyanide or potassium permanganate and then treated with Marquis' reagent, a blue color is immediately obtained. (With Marquis' reagent alone, papaverine gives practically no color at first; after a little time a purple-red develops.) Warren tried out a considerable number of other alkaloids, including pseudomorphine and other opium alkaloids, but reported a similar effect only with an unnamed alkaloidal substance from *sanguinaria*. He did not mention the corresponding reaction of pseudomorphine¹.

In 1926 J. Aloy and A. Valdiguié reported a new test for codeine. The original article is not available to the writer, and he trusts that he does these workers no injustice when he quotes the abstract, since the misconception of the reaction is apparent:

To identify *codeine*, dissolve a particle of the alkaloid in 3-4 cc. pure H_2SO_4 , add 2 drops of a 1 per cent $UO_2(AcO)_2$ soln or of very dil. soln of $Fe(AcO)_3$ or other ferric salt (which should not by itself color the codeine soln), then add 1 drop of $HCHO$ of 1 : 10,000 diln; the blue color formed within 2 min., indicates codeine. Formol at this diln does not react with the codeine in absence of the catalyser².

The impression given is that the formaldehyde reaction with codeine is "catalysed" by a heavy metal, uranium or iron. This is almost the reverse of the fact, for if there is anything that can be called catalysis in the reaction, it is the formaldehyde which catalyses the oxidation reaction.

In 1928 the writer published an article in which he introduced two new reagents, made empirically with nitric acid, formaldehyde, and sulfuric acid, calling them "nitric-acid formaldehyde reagents" and distinguishing them as Reagents "A" and "B"³. They give very striking reactions, quite different from those of Marquis' reagent, not only with pseudomorphine and papaverine, but also with morphine and codeine, the codeine reaction with Reagent "A" and both morphine reactions being unlike any previously reported for these alkaloids.

The method of adding the formaldehyde and oxidizing agent successively has now been developed, but not all the effects resulting from their incorporation in a single reagent can be duplicated exactly.

METHOD OF MAKING THE TESTS.

The tests on the opium alkaloids are carried out as follows:

Put a little of the alkaloid on a spot plate and add 0.4-0.5 cc. of the solution of formaldehyde in sulfuric acid, just about filling the "spot". Stir with a glass rod, getting the alkaloid into solution and noting any colors that may be produced. To observe the effect of the formaldehyde alone simply allow this solution to stand; to

¹ *J. Am. Chem. Soc.*, 1915, 37: 2402.

² *J. pharm. chim.*, 1926, 4: 390; *C. A.*, 21: 2358.

³ *J. Lab. Clin. Med.*, 1928, 13: 750.

observe the effect of the formaldehyde plus an oxidizing agent, add a single drop of one of the oxidizing solutions (given below) immediately after stirring the alkaloid-formaldehyde solution, and then stir the solution again.

The alkaloid, preferably, should be free or in the form of the sulfate. The use of the hydrochloride introduces a complication in that the oxidizing agent may liberate chlorine and so alter the reaction. The hydrochloride often gives a somewhat different reaction from the sulfate with concentrated sulfuric acid reagents that have an oxidizing action.

A strong bright color is usually produced at once, but the solution should be kept under observation for about 15 minutes, or even half an hour. Changes occurring or colors developing too slowly to be observed in this time are of little value and are too likely to be due to the absorption of some active substance from the laboratory air.

In general heroine reacts like morphine and dionine like codeine in these tests, therefore no references to them will be made except in the one case in which morphine is distinguished from heroine.

THE OXIDIZING SOLUTIONS.

The four following oxidizing solutions have been found to be useful:

(1) *Bromine oxidizing solution*.—While cooling with running water, mix 0.5 cc. of bromine water with 3.5 cc. of concentrated sulfuric acid.

(2) *Ferric oxidizing solution*.—Mix 1 cc. of 10 per cent aqueous ferric sulfate solution with 3 cc. of concentrated sulfuric acid, cooling.

(3) *Nitric acid oxidizing solution*.—Prepare very dilute nitric acid by mixing 5 drops of concentrated nitric acid with 50 cc. of water. For the oxidizing solution mix 1 cc. of this dilute nitric acid with 3 cc. of concentrated sulfuric acid, cooling.

(4) *Stronger nitric acid oxidizing solution*.—Prepare dilute nitric acid by mixing 1 cc. of concentrated nitric acid with 30 cc. of water. Mix 1 cc. of this dilute nitric acid with 3 cc. of concentrated sulfuric acid, cooling.

The solutions are given in order of their strength. The ferric oxidizing solution is the most useful with concentrated sulfuric acid, the nitric acid solution with acid that is not full strength. The others have special uses only.

THE REACTIONS OF THE OPIUM ALKALOIDS.

Marquis' Reagent Followed by an Oxidizing Agent.

Marquis' reagent consists of 2 drops of 37 per cent formaldehyde solution added to 3 cc. of concentrated sulfuric acid. It must be free from any oxidizing action, and even though the acid has been previously tested, it is best to test the reagent itself on pure brucine, with which it should give no color. It gives the following color reactions with the

opium alkaloids: Morphine, crimson changing to purple; codeine, purple; pseudomorphine, red; apomorphine, purple changing to dark green; papaverine, a delayed purple-red; narcotine, purple, after a little fading and changing to brown, then yellow; narceine (with which pure sulfuric acid strikes brown and gives brownish yellow on solution), soon develops orange changing to red; thebaine, same as with pure sulfuric acid, which strikes brown-red and gives an orange solution.

When Marquis' reagent is followed by the bromine oxidizing solution the pseudomorphine red changes to a beautiful strong purple, and then gradually to deep blue. The morphine and codeine reactions are also changed, morphine giving violet changing to purple-red, and codeine giving deep blue. The reactions of the other alkaloids are but little affected. When Marquis' reagent is followed by the ferric oxidizing solution, the noteworthy changes in the formaldehyde colors are as follows: Morphine, blue, rather slowly changing to dull purplish red; codeine, blue, gradually dull bluish green; pseudomorphine, blue; papaverine, blue; thebaine, the orange rather slowly changes to olive or even green.

The combination of Marquis' reagent with the nitric acid oxidizing solution is especially useful for thebaine. A deep bluish green color is produced, which soon becomes a persistent deep bright green. Very little thebaine should be used. Papaverine gives blue and pseudomorphine blue-green. Marquis' reagent followed by the stronger nitric acid oxidizing solution gives with thebaine a deep green color, which soon fades to light orange; and with pseudomorphine a remarkable reaction, blue, changing quickly to purplish red, then to brown, and finally to green. The other alkaloids are over-oxidized and so do not give useful reactions.

Dilutions of Marquis' Reagent with Concentrated Sulfuric Acid.

A 20-fold dilution of Marquis' reagent with pure concentrated sulfuric acid shows the changes produced in the reactions as the proportion of formaldehyde is reduced. Pseudomorphine still develops a red color gradually; the brownish yellow of narceine rather slowly changes to red; and narcotine gives greenish yellow changing to purple. These alkaloids, therefore, still show weakened Marquis' reactions. Morphine, however, gives light yellow slowly changing to dark blue; codeine, orangish yellow slowly changing to dark green; and apomorphine a brownish color, changing to dark green.

On adding the ferric oxidizing solution immediately after the alkaloid is dissolved a very peculiar effect is observed with apomorphine, morphine and codeine, in that it seems to "bring back" to some extent the original Marquis' reaction. Apomorphine gives bright purple changing to dark green quickly; morphine gives a purplish color, changing to weak

gray blue at once, and then to blue-green; codeine gives a momentary purple changing to bright green, then to dark green, and finally to violet-blue-black, or, if the reagent is diluted a little more (say 30-fold), the dark green changes to deep violet. Pseudomorphine gives greenish blue; papaverine, blue; and the orange shown by thebaine gradually becomes green.

A 300-fold dilution of Marquis' reagent with pure concentrated sulfuric acid gives only slight indications of the presence of formaldehyde when applied to the opium alkaloids. Codeine, morphine, and apomorphine give a yellowish color, which becomes greenish; narcotine gives yellow as with pure sulfuric acid; narceine and thebaine also give their sulfuric acid reactions. The addition of a drop of the ferric oxidizing solution quickly produces greenish blue changing to green with pseudomorphine; purple changing to blue-gray and then to purple-red with apomorphine; and blue with papaverine. Morphine develops a purple-red like the final apomorphine color; codeine develops violet; and the thebaine color gradually changes to olive or green.

A 1000-fold dilution of Marquis' reagent followed by the ferric oxidizing agent gives results similar to those just described. Apomorphine, however, does not show a succession of colors, but simply strong deep purple.

Some mention should perhaps be made of the action of the ferric oxidizing agent when no formaldehyde at all is present. With pure sulfuric acid and a drop of the oxidizing agent apomorphine gives dull blue, and pseudomorphine rather weak dull green, after a time changing to brown. Narcotine gives the usual yellow, but this changes slowly to orange-red as oxidation takes place. The other alkaloids show no color changes in the cold.

Dilutions of the Acid.

When the strength of the acid is reduced, the Marquis reactions of narcotine, pseudomorphine, papaverine, and narceine are soon lost. A new test for narceine, however, of the aldehyde-oxidation type, can be obtained by using sulfuric acid of 85-88 per cent strength.

To 5-6 cc. of concentrated sulfuric acid add 1 cc. of water, mix and cool. Add 4-7 drops of 37 per cent formaldehyde solution. This reagent gives strong Marquis' reactions with morphine, codeine, and apomorphine; in fact the apomorphine reaction is better than when the full strength acid is used. Thebaine and narceine react about the same as with pure concentrated sulfuric acid, while narcotine, papaverine, and pseudomorphine give practically no reactions.

If this reagent, a "Marquis reagent" made with 86 per cent sulfuric acid, is followed by the nitric acid oxidizing solution, narceine gives a good dull greenish blue. (Omitting the formaldehyde, narceine and the

oxidizing agent in this strength acid gives a weak gray blue.) Papaverine gives blue and pseudomorphine gives green-blue changing to bluish green.

The reactions with only a trace of formaldehyde are also of importance. Dilute a little 37 per cent formaldehyde solution 1000 times with water. Add 1 drop of this solution to each cc. of 86 per cent acid taken for the reagent. This solution, followed by the nitric acid oxidizing solution, gives with codeine or papaverine, blue; with pseudomorphine, blue-green; with apomorphine, purple changing to purplish red; and with morphine, dull green changing to brown.

Some valuable results are obtained with still weaker acid, about 75 per cent sulfuric acid. Dilute 2 cc. of concentrated sulfuric acid with 1 cc. of water and add 3-4 drops of 37 per cent formaldehyde solution. Morphine, codeine and apomorphine still give their Marquis' reactions, but heroine now gives no color at first and reacts only as it is hydrolyzed to morphine. The test can be used to distinguish morphine from heroine, and even to detect morphine in the presence of heroine. Thebaine still gives its sulfuric acid reaction, while narcotine and narceine give no colors.

For the combination reactions, dilute 37 per cent formaldehyde solution 300 times with water, and add 1 drop of this solution to each cc. of the 75 per cent sulfuric acid taken for the reagent. Follow this reagent with the nitric acid oxidizing solution. Codeine gives blue, pseudomorphine blue-green, and apomorphine purple; the other combination reactions are lost.

"COMBINATION" REAGENTS.

In nearly every case the reactions previously considered can be obtained by combining into one reagent certain proportions of formaldehyde and an oxidizing agent. The single reagent may also give effects, particularly with morphine and codeine, not readily obtained by adding the two ingredients separately. This is the case with the original reagents of this type used by the writer, "A" and "B".

The following new reagents give reactions similar, respectively, to those given by Reagents "A" and "B", and they are easier to make and more stable.

Reagent "C".—To 6 cc. of Marquis' reagent add 0.8 cc. of 10 per cent aqueous ferric sulfate solution, and mix while cooling.

Reagent "D".—Dilute Marquis' reagent about 800 times with concentrated sulfuric acid. To 6 cc. of this solution add 0.8 cc. of 10 per cent aqueous ferric sulfate solution, and mix while cooling.

These reagents give reactions as follows:

Reagent "C": Morphine—purple-red, instantly changing through blue-green to dark blue, then changing to violet, then to red, and slowly

to brown-green; codeine—deep bright green, gradually changing to dark greenish blue, slowly to brown; pseudomorphine—blue green changing to greenish blue; papaverine—blue.

Reagent "D": Morphine—slight gray blue changing to stronger violet, then to purple-red; codeine—greenish color, soon changing to deep blue, gradually to purplish blue; pseudomorphine—green; papaverine—blue.

ALDEHYDE-ALKALOIDS.

Both cotarnine and hydrastinine contain an aldehyde group, and react as aldehydes with phenols in the presence of concentrated sulfuric acid. Some other alkaloids decompose with concentrated sulfuric acid to give aldehydes. Narcotine, narceine, and hydrastine yield opianic acid, an aldehyde, on hydrolysis. On hydrolytic oxidation narcotine gives both opianic acid and cotarnine, while hydrastine gives opianic acid and hydrastinine. The alkaloids which yield aldehydes in the reaction that is considered in this paper may be called aldehyde-alkaloids. Pseudomorphine in particular gives strong reactions with them. They may be tested as follows:

Mix a little narcotine and pseudomorphine on the spot plate, dissolve in concentrated sulfuric acid, add a drop of the nitric acid oxidizing solution, and stir. A strong bright green color results.

The test is sensitive to both alkaloids. Narceine, which is closely related to narcotine, gives a similar test with pseudomorphine; cotarnine, hydrastinine, and hydrastine give even stronger blue-green colors.

Other phenols may be substituted for pseudomorphine and corresponding reactions obtained.

SUMMARY.

(1) New tests are given for thebaine, narceine, pseudomorphine and apomorphine, and these are coordinated with tests previously reported for pseudomorphine, papaverine, codeine and morphine.

(2) These tests are particular cases of a general reaction: the oxidation of the compound formed by a phenol (or phenolic ether) and an aldehyde in strong acid. They are aldehyde-oxidation tests for phenols.

(3) Reactions of this type should be of value, not only for the identification of the opium alkaloids, but also for the identification of other phenols and phenolic compounds, for the detection and identification of formaldehyde, and for the identification of substances as aldehydes, or as compounds yielding aldehydes with strong acid.

NOTE.

The Immersion Filter for Phosphorus, Calcium and Crude Fiber Determinations¹.

One of the unique uses of the Jena glass filter plate² is its employment in an immersion filter. This new device offers a departure in the process of filtering troublesome precipitates as well as the complete washing of crude fiber residues entirely within the original flask. The precipitate may be easily dissolved from the filter, which leaves the solution free from obscuring media or apparatus for the titration. The immersion filter is entirely of glass, always assembled ready for use, and it can be used with the usual glassware and tubing found in any laboratory.

Description and manipulation.—An immersion filter of 180 mm. height may be used with a wide-mouth 250 cc. Erlenmeyer flask for phosphorus or calcium precipitates or with one of 500 cc. capacity for digesting crude fiber. For fine precipitates of a few milligrams of calcium or phosphorus the porosity should be 4–5 μ , but larger porosity may be safely used for filtering crude fiber. It is essential to have a filter of zero capacity so that all the filtrate, as well as each successive wash, is withdrawn from the precipitate. The vacuum should be carefully adjusted so that the film is not broken in the disc or loss of fine precipitates may result; greater vacuum may be used with crude fiber filtration. The solution of the precipitates is easily made by adding a few cc. of the solvent through the stem of the filter and forcing it through the disc by pressure from an attached rubber bulb. A few washings of hot water will clear the disc and clean the tube of solvent if the determination is to be finished by titration.

Phosphorus determinations.—The precision of the immersion filter was compared with the adopted Shimer tubes by the same method as was used by the writer³ for the glass crucibles. The mean of a set of 10 determinations, containing 4.37 mg. of phosphorus each, was 4.26 mg., with a probable error of ± 0.07 mg. of phosphorus for the immersion filter. The mean of 10 determinations of like concentration for Shimer tubes was 4.25 mg., with a probable error of ± 0.02 mg. of phosphorus. This indicates that the Shimer tubes are more precise for small quantities of phosphorus, even though the immersion filter greatly reduces the time.

Calcium determinations.—Calcium was determined as calcium oxalate from a solution containing 4 mg. of calcium per determination by the immersion filter. The mean of 10 determinations was 3.96 mg. of calcium with a probable error of ± 0.03 mg. of calcium.

¹ Contribution from the Ohio Agricultural Experiment Station, Wooster, Ohio, by R. H. Simon.

² Huttig, *Z. angew. Chem.*, V. 37: 48, and Nette, *Z. anal. Chem.*, 1925, 65: 385.

³ *This Journal*, 1929, 12: 209.

Crude fiber determinations.—The mean of 10 determinations of crude fiber filtered by the immersion filter was 453.6 mg. with a probable error of ± 0.22 mg. on a single determination. The use of asbestos is unnecessary because the crude fiber can be washed free of acid or alkali with the flask. In addition to the uses mentioned in this paper the immersion filter has been found valuable for filtering extracts of plant residues from cellulose-dissolving solvents, such as ammoniacal cupric hydrate, which cannot be safely accomplished by paper or asbestos.

BOOK REVIEWS.

Photometric Chemical Analysis (Colorimetry and Nephelometry). Vol. II. **Nephelometry.** By John H. Yoe, Ph.D., Professor of Chemistry, University of Virginia. With contributions by Hans Kleinmann, M.D., Ph.D., University of Berlin. John Wiley & Sons, Inc., New York, 1929. XVI + 337 pp., 44 figs. 6 x 9 ins. Price \$4.50.

In this volume the facts of nephelometry have been ably summarized for advanced students and research workers. In its preparation the author had in mind a six-fold purpose: "(1) To give an accurate account of the development of nephelometry, (2) to present an impartial discussion of the present status of the theory of nephelometry, (3) to give detailed working directions for using a precision nephelometer, (4) to discuss nephelometric research, (5) to give procedures for the determination of a number of inorganic and organic constituents, and (6) to give an accurate and fairly complete survey of the literature on nephelometry".

The first four items are discussed in Part I. The use of calibration curves is emphasized; such curves are frequently illustrated, and for the convenience of the worker numerous cross-section sheets have been included at appropriate places throughout the procedures. Parts II and III consist of methods for the determination of inorganic and organic constituents, respectively; the former includes procedures for ammonia, arsenic, calcium in the blood and in milk, chlorine as chloride, phosphorus in urine and iron and as phosphoric acid, and sulfur in blood and in urine; the latter, acetone, anilase, dichloro-ethyl sulfide, fats, lipase, nucleic acids, β -oxybutyric acid, pepsin, proteins, purine bases, including uric acid, and trypsin. The bibliography contains over two hundred references to the literature, arranged alphabetically, and with each reference a summary of the important points of the investigation is given.

This volume has been appropriately dedicated to the memory of Theodore William Richards as the founder of nephelometry. Two of Dr. Richard's letters have been reproduced.

Errata for Vol. I have been inserted in this volume.—W. L. HILL.

NEW BOOKS.

Dairy Bacteriology. By B. W. Hammer. 473 pages. John Wiley & Sons, Inc., New York, 1928. Price \$5.00. Bacterial counts; milk fermentation; contamination and its reduction; growth of microorganisms; body cells; spread of diseases through milk and its derivatives; preservation of milk and cream; enzymes; bacteriology of evaporated and sweetened condensed milk, and powdered milk, ice cream, butter cultures, fermented milks, butter and cheese; and test for quality of milk and cream are the subjects treated.

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